Factors associated with the occurrence of Developmental Defects of Enamel and Dental Fluorosis among 4 and 8 year olds in Nigeria

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Abstract

Enamel formation may be affected by genetic and environmental factors resulting in enamel defects such as developmental defects of enamel (DDE) and dental fluorosis. This 3 phase project investigated the influence of fluoride (F) exposure (intake and excretion), nutritional deficiencies, dental factors as well as other environmental factors and genetics on DDE and dental fluorosis among 4 and 8 year old Nigerian children.

In Phase 1, dental examination for the presence of DDE, dental fluorosis and caries experience using the modified DDE index, Thylstrup and Fejerskov (TFI) and Dean's indices and dmft/DMFT indices respectively was undertaken for 302 four-year-olds and 322 eight-year-olds and a parent/guardian questionnaire collected data on dental health and nutrition. In Phase 2, information and samples to estimate F intake and urinary F excretion of a sub-sample of 61-four- and 64 eight-year-olds was obtained. In Phase 3, a buccal mucosa swab was collected from the subsample for gene sequencing to determine the presence of Single Nucleotide Polymorphisms (SNP) in the COL1A2 gene.

The prevalence of dental fluorosis, dental caries and DDE was 5.6%, 10.6% and 78.5% and 9.3%, 16.8% and 64.7% in the primary dentition of 4 and 8 year olds respectively while it was 29.8%, 7.5% and 67.1% respectively in the permanent dentition of 8 year olds. The Spearman correlation coefficient for severity of primary dentition dental fluorosis and drinking water F in 4 and 8 year olds was 0.12 and 0.15 respectively ($p<0.05$); in the permanent dentition of 8 year olds it was 0.17 ($p=0.002$) for cooking water F and 0.28 ($p=0.001$) for drinking water F. The Pearson correlation coefficient for Total Daily F Intake (TDFI) and urinary F excretion was 0.41 ($p=0.001$) and 0.57 ($p<0.001$) in 4 and 8 year olds respectively. Drinks, foods and toothpaste contributed 17%, 54% and 29% and 21%, 54% and 25% to TDFI among 4 and 8 year olds respectively. Fluoride toothpaste use, gender, drinking water F, cooking water F, exclusive breastfeeding, infant/childhood diseases, TDFI and Total Daily Fluoride Retention (TDFR) were statistically significant predictors of DDE and dental fluorosis ($p<0.05$). The presence of SNPs CC and AC in the COL1A2 gene approached statistical significance as predictors for dental fluorosis ($p=0.08$).

Enamel formation is vulnerable process, prone to many influences. The DDE and TFI indices provided a sensitive record to explore predictors for the presence of enamel defects and dental fluorosis in this young Nigerian population. Adoption of oral health and feeding habits which prevent excessive F exposure remain key principles to mitigate against these conditions.

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Chapter 1 Introduction.

1.1 The Federal Republic of Nigeria and its people

Nigeria, the tenth largest country in the world and the fourth largest economy in Africa, lies on the west coast of Africa at geographical coordinates of 10^{o} North and 8^{o} East and occupies approximately 923,768 km^2 of land bordering Niger, Chad, Cameroon and Benin (Odujinrin, 2009). Nigeria is the most populous nation in Africa with a population of 165, 471,000 in 2011 and GDP p.c. growth of 2.1% p.a. between 1990 and 2011 (Commonwealth Year Book, 2013). The population of Nigeria is unevenly distributed with large areas in the Chad basin, middle Niger Valley, grass plains of Oyo and Niger Delta sparsely populated while extensive areas in parts of the south and north are densely populated (National Population Commission, 2010). About 64% of the Nigerian population lives in rural areas while the balance of 36% lives in urban areas (Odujinrin, 2009). Between 1900 and 1914, Britain governed Nigeria as two separate protectorates: the Northern and southern protectorates (All Nigeria History, 2012). In 1914, Governor-General Frederick Lugard amalgamated these two British protectorates to become Nigeria, ruled by a single central colonial government (All Nigeria History, 2012, Daniels, 2012). After the amalgamation, these protectorates were divided into three main regions: north, west and east and within these regions there were sub-regions categorized based on history, geography and ethnicity (Daniels, 2012, Commonwealth Year Book, 2013). Nigeria became an independent country on the 1st October, 1960 after about a century occupation by the British colonial masters (George et al., 2012) and became a republic in 1963 (Olumide and Ekanade, 2011). To make governance easy, Nigeria is administratively divided into 36 states and the Federal Capital Territory (FCT) (Ofem, 2012, Nigeria Demographic Health Survey, 2013), while these 36 states are divided into six geopolitical zones (Eze et al., 2014). These zones are at located at varying altitudes, ranging between 38-302 meters in the south of Nigeria and 115-645 meters in the northern part of Nigeria (Akpata et al., 2009) as shown in Table 1-1:

Geopolitical zone	Altitude (m)
North Central	115-467
North East	190-609
North West	351-645
South South	38-98
South East	91-137
South West	224-302

Table 1-1: Geopolitical zones in Nigeria at varying altitudes.

Figure 1-1: **Map of Nigeria showing the states and the federal capital territory.**

Each state in Nigeria comprises 3 senatorial districts (Akpata et al., 2009) and each senatorial district is divided into Local Governments Areas (LGAs) (Olusile et al., 2014). Altogether, there are 774 LGAs in Nigeria (Olusile et al., 2014). Nigeria is composed of more than 250 ethnic groups (Gandonu, 1978) however it is made up of three major ethnic groups, namely, Hausa and Fulani; Yoruba and Igbo (Langer and Ukiwo, 2007). The official language of communication is English, which is taught in primary schools and used for instruction in secondary schools and universities (Danladi, 2013). In addition to

English, pidgin has been used as a lingua franca especially in the south of Nigeria (Onyeche, 2004). Likewise, there are over 500 additional indigenous languages in Nigeria thereby making many Nigerians trilingual. Some Nigerians, aged 15 – 49 years have no formal education, 63.0% and 79.0% of females and males are literate respectively and the literacy rate is higher in urban (71.0%) than in rural areas (47.0%) (National Population Commission and ICF Macro, 2009). There are several religions in Nigeria which indicate ethnic and regional differences. The north of Nigeria where the majority of the inhabitants are Hausas and Fulani's is dominated by Muslims, Catholicism is mainly practiced by the Igbos in the South-east and Protestantism and local syncretic Christianity were mostly practice by the Yorubas in the South-west of Nigeria.

1.1.1 The geography of Nigeria

Nigeria has two main navigable rivers namely: the River Benue and the River Niger from where the country derives its name. The River Niger drains to the south of the region into the Gulf of Guinea via Africa's largest delta which is about 100 km wide. The coastal region of Nigeria is low-lying and has sandy beaches, lagoons and mangrove and fresh water swamps (Commonwealth Year Book, 2013). Nigeria is made up of three major geological terrains namely: the crystalline basement complex, the Jurassic younger granites and the sedimentary terrain (Lar and Tejan, 2008). The crystalline basement complex which is Precambrian in age and form the pre-existing bedrock comprises the gneiss-migmatities, schist belts and older granites (Lar and Tejan, 2008). The sedimentary terrain contains sediments and is most commonly found in southern Nigeria and the Lake Chad basin in the north east (Akpata et al., 2009). The crystalline basement complex crops out more extensively in northern Nigeria. Much of Nigeria's surface consists of ancient crystalline rocks of the African Shield which have been subject to weathering and erosion for long periods resulting in a characteristic landscape of extensive level plains interrupted by occasional granite mountains (Helen, 1991). To the north of the Niger and Benue basins, these granite mountains characterized most part of northern Nigeria together with broad and stepped plateau. These types of mountains are also found between coastal plains and the upper Niger Basin and there are also smaller areas of younger granites found in some locations such as Jos Plateau (Helen 1991). Similarly, in many areas of Nigeria, sedimentary strata consisting of flat-topped ridges and dissected plateaus and rocky outcrops lie over older rocks. These mountains, hills, plains and plateaus are scattered all around the country. There are some places in Nigeria that have witnessed volcanic activities

namely Biu Plateau in the northeast and Jos Plateau in the north central region of Nigeria (Helen 1991).

In Nigeria, the environment is an entire web of geological and biological interactions resulting in trace elements finding their way into the human body directly from soils and/or the underlying bedrock or through plants and the general food chain (Lar and Tejan, 2008). Fairbride (1972) reported that some large and selective concentrations of metals originating from the underlying bedrock are found in certain plants. This could affect humans either positively or negatively since most of the population of Nigeria depends on the land for their daily activities. The most important and significant environmental problems in Nigeria are rapid deforestation, soil degradation and desertification.

1.1.2 The climate of Nigeria

As in most of West Africa, Nigeria's climate is characterized by strong latitudinal zones, becoming progressively drier as one moves north from the coast (Helen, 1991). There are three periods of the year in the north namely: the dry harmattan period from November-February, the hot dry period from March-May and the hot, humid and wet period from June-September. In the south there are basically two periods of the year namely: the dry season which starts from November to March and the wet seasons which starts from April to October.

Nigeria has humid tropical climate and is warm with little variation in temperature which is mostly between 28-32^{o}C throughout the year (Akpata et al., 2009). This is because the country is situated just north of the equator. In most part of southern Nigeria, the wet season goes from around March/April to October/November; with a mean annual rainfall of about 1,200-1,300 millimetres while the rainy season usually starts in June/July in most parts of northern Nigeria and may last for only 3-5 months with a mean annual rainfall of 500-750 millimetres (Akpata et al., 2009).

1.1.3 Nutrition in Nigeria

The food consumption patterns of Nigeria can be associated with changes in her demographic and socio-economic environment (Omotor, 2009). Changing food consumption patterns have both positive and negative health consequences (Lopriorea and Ellen, 2003). The nutritional status of the average Nigerian remained precarious as the country consistently records a deficient or low average per capita calorie intake (Olayiwola et al., 2004, Orewa and Iyangbe, 2010). The low level of government budgetary allocation

to both health and education is also a clear indication that priority is not placed on activities that have direct links with health and nutritional status. Nigeria imports food to complement local production and the majority of people consume street foods and foods from fast food chains. This is because of their quest for and easy means of getting food at any time of the day. In the far north and the Niger Delta, food security is a major concern because household source of food and money have significantly reduced as prices of food remain high (Omotor, 2009). The pattern of food consumption in Nigeria varies; however, the majority consumes dietary fibres (Mbofung and Atinmo, 1984, Olayiwola et al., 2013). A study on micronutrient status and nutrient intake of elderly Nigerians reported that the majority were deficient in vitamins and minerals (Olayiwola et al., 2014). Pregnant women in Sub-Saharan countries like Nigeria are at risk of poor nutritional status and adverse outcomes as a result of poverty and food insecurity (Lindsay et al., 2012). Nutritional deficiencies such as micronutrient deficiencies are common during pregnancy especially among pregnant women from economically disadvantaged settings in Nigeria where diets with low density of minerals and vitamins are consumed (Ugwuja et al., 2011, Ejezie and Nwagha, 2011).

Breastfeeding especially when used as an exclusive form of nutrition in neonates and infants is central and key to child survival in developing countries like Nigeria. However, tradition and/or women's economic responsibilities as well as early introduction of complementary feeding has led to low rates of exclusive breastfeeding (EBF) (Olayiwola et al., 2004, Ugboaja et al., 2013). Although 96 percent of infants in Nigeria are breastfed, the median duration of EBF is less than one month and that of full breastfeeding is just over two months (Agho et al., 2011). The urban rates of breastfeeding infants within an hour or a day of birth are higher than the rural rates (Sadoh et al., 2011). However, infants are breastfed longer in rural than urban areas (Federal Office of Statistics, 2000).

In Nigeria, protein energy malnutrition is the second most significant cause of morbidity and mortality among children after infections (Uthman, 2009). Reports have shown that many children in Nigeria are stunted and wasted and stunting and malnourishment is more prevalent in rural than urban areas (Federal Government of Nigeria et al., 2003, Maziya-Dixon et al., 2004, Adekanmbi et al., 2013). Maternal under-nutrition has a close link with low birth weight and childhood stunting and underweight (Olayiwola et al., 2004, Senbanjo et al., 2013). Three key micronutrient deficiency disorders, vitamin A deficiency (VAD), iron deficiency anaemia (IDA) and iodine deficiency disorders (IDD) are common in many parts of Nigeria (Federal Ministry of Health and Social Services and USAID, 1993).

Micronutrient deficiency rates also vary along the urban/rural continuum, according to Federal Government of Nigeria et al. (2003). Gegios et al. (2010) reported that in Nigeria, 17.0% of children had adequate intake of vitamin A, 57.0% iron and 41.0% zinc and consumption of cassava is a risk factor for inadequate micronutrients when a 24 hour dietary recall of children was done. Table 2 shows the micronutrient deficiency among under 5-year-old children by location.

Micronutrients	**Percentage deficiency**			
	National	**Rural**	**Medium[1]**	**Urban**
Vitamin A	29.5	25.6	32.6	25.9
Vitamin E	22.6	21.5	26.3	17.8
Iron	27.5	24.4	27.9	33.1
Zinc	20.0	26.0	17.0	17.0
Iodine	12.2	15.5	10.6	10.6

Table 1-2: Micronutrient deficiency among under 5-year-old Nigerian children by location. *Source: (Maziya-Dixon et al., 2004).* *Notes: [1] – Semi-urban*

Nigeria is undergoing a dietary change, with more people craving for westernized diet. This has led to an increase consumption of free sugars especially among children and adolescents (Onyiriuka et al., 2013) with its attendant consequences of dental caries (Oziegbe and Esan, 2013). The proportion of people who consumed these free sugars is more in urban communities than in rural communities (Okeigbemen, 2004) and anecdotally it has remained so. This might be the reason why the prevalence of dental caries is higher in urban communities than in rural communities.

1.1.4 Water services in Nigeria

The provision of water supply is the responsibility of the federal, state and local governments however, only small portion of the demand have been met. The majority of people living in urban and semi-urban communities have no reasonable access to reliable water supply. This has led to many people purchasing water from private vendors in cans, bottles and sachets which are more expensive than from public supply. In addition, people tend to sink boreholes and wells especially in private homes; however, there are few locations where people get their water from community wells. Nigeria has adequate surface and ground water resources to meet current demands for potable water. Rapid depletion of ground water especially in the north and insufficient control of water pollution and serious erosion problems in the south have worsen the problem of water supply. There are linkages between water supply and sanitation and sectors such as health, agriculture, environment, education, enrollment in schools, poverty and productivity (Idowu et al., 2012).

Fluoride concentrations in water sources vary widely within Local Government Areas in Nigeria, thus there may be no clear cut boundaries to the geographical distribution of fluoride levels. However, the fluoride map (Figure 2) by Akpata et al. (2009) provides some indication of the distribution of fluorides in drinking water in different parts of Nigeria.

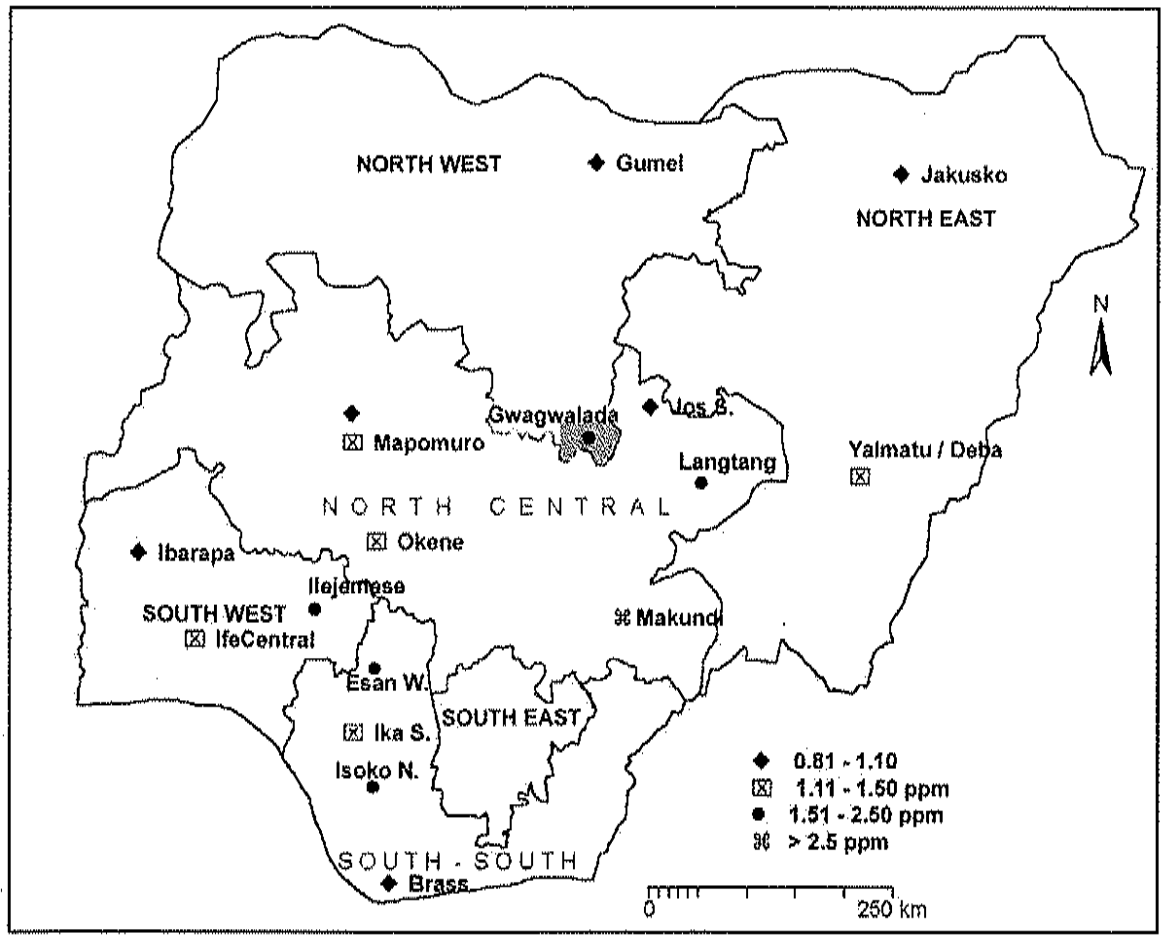

Figure 1-2: Map of Nigeria showing Local Government areas with drinking water containing fluoride levels higher than 0.8 ppm in the six geopolitical zones. Source: Akpata et al. (2009).

The map showed that fluoride concentration was above 1.5 ppm in a number of places in the country namely; Langtang in Plateau State, North Central geopolitical zone; Isoko North in Delta State and Esan West in Edo State, South-South geopolitical zone and Ilejemese in Ekiti State, South West geopolitical zone. Generally they reported that fluoride levels were significantly higher in North Central geopolitical zone than in other zones; the highest fluoride concentration of 6.7 ppm was recorded at a deep well in a location in Makundi (Figure 1.2) Benue state north central Nigeria. Fluoride levels were lowest in the

South East zone. Akpata et al. (2009) reported that the relatively high concentrations of fluoride in drinking water in North Central geopolitical zone might be related to the igneous and volcanic rocks found in the Jos Plateau. In Nigeria, less than 10.0% of the drinking water sources were from waterworks, the majority gets their water from boreholes, wells and rivers/streams therefore artificial fluoridation of pipe-borne water would benefit only a small segment of the Nigerian community (Akpata et al., 2009).

1.1.5 The health services in Nigeria

In the early part of the 19th century, medical services were mainly provided by traditional medicine practitioners and it was not until the 1860s that western medicine was introduced by the establishment of the Sacred Heart Hospital by Roman Catholic missionaries in Abeokuta, southwestern Nigeria (Helen, 1991). Modern health care facilities were provided by religious missions throughout and after the colonial period and many of these health facilities still remain important parts of the health care systems in Nigeria providing medical training and education (Helen, 1991). In many rural and urban communities in Nigeria, there has been an increased improvement of public health services, growth of medical education, establishment of community health centres and control of disease vectors and contagious diseases. In the late 1980s, government health policies focused on a significant growth in community health centres with increased vaccination against major childhood diseases (Helen, 1991). However, there is inequality in the distribution of health facilities among regions, urban and rural areas and socio-economic classes.

In 2011, the public spending on health was 5.8% of the GDP, the infant mortality rate was 78 per 1000 live births, the maternal mortality rate was 840 deaths/100,000 live births and the life expectancy at birth in Nigeria was 47.56 years (United Nations World Population Prospects, 2010). The ratio of physician to population is 0.395 physicians/1,000 population and hospital bed density is 0.53 beds/1,000 population (United Nations World Population Prospects, 2010).

1.1.6 Oral health care in Nigeria

In Nigeria, dental treatments are provided by few dentists in both private and public dental clinics but the majority of the clinics are located in urban communities. There are also few dental hygienists or therapists who work under the supervision of the dentists and occasionally they visit rural communities since the rural population has little or no access to modern dental treatment. There appears to be no coordinated national approach to

preventive and therapeutic oral health services and the provision and utilization of oral health care services in Nigeria is poor accounting for the increase prevalence of oral diseases. Periodontal disease and dental caries are the two major oral health problems while others include malocclusion, traumatized anterior teeth, oral tumors, developmental defects of enamel and dental fluorosis (Akpata, 2004a, Ogini and Adekoya-Sofowora, 2007, Orenuga and Odukoya, 2010, Danielson and Chinedu, 2011, Gbadebo, 2012). The prevalence of periodontal disease is quite high and the occurrence of the disease is related to oral hygiene status and socio-economic class (Adegbembo and El-Nadeef, 1998, Orenuga and Odukoya, 2010). A recent study (Sofola et al., 2014) on dental caries among primary school children in Lagos Nigeria reported an increase in caries prevalence in the primary dentition. Studies on the occurrence of developmental defects of enamel and dental fluorosis in Nigeria are sporadic and these studies reported variations in their prevalence. There is very limited information on factors responsible for the occurrence of developmental enamel defects in Nigeria.

1.2 Background for this study

Developmental defects of enamel such as enamel hypoplasia and opacities and dental fluorosis are one of the anomalies of enamel formation seen among people of various races and tribes. These defects present important clinical significance since they are responsible for aesthetic problems, dental sensitivity, erosion, wear, dentofacial anomalies as well as predisposition to dental caries. The aesthetics problems can affect the development of self-esteem in an individual especially children. The prevalence and severity of these developmental enamel defects varies across and within countries. Similarly, the pattern of distribution of the defects also varies among several population groups and the variation is observed in the dentitions, the jaws and among both gender. The variation in the prevalence, severity and distribution of developmental enamel defects could be due to the population studied, the teeth examined and the criteria used to diagnose developmental enamel defects. This difference could also be due to several factors since the defects are caused by prenatal and postnatal interactions of several environmental and intrinsic or endogenous factors such as genetics. These factors may influence the activity amelogenesis resulting in ameloblasts laying down of defective enamel. Several factors such as socio-economic status, trauma to tooth buds, childhood illnesses, drugs, chemicals, altitude, nutritional problems and problems during pregnancy have been implicated in the aetiology of developmental enamel defects. Fluoride, a chemical agent when excessively ingested may result in dental fluorosis; however, when it is optimally ingested it has been found to

have an inverse relationship with the prevalence and progression of dental caries thereby playing significant role in dental caries prevention. Therefore considerable research efforts have been directed towards assessing optimum levels of fluoride intake that are compatible with dental and general health in diverse populations.

In Nigeria, the few studies on developmental defects of enamel were on prevalence and pattern of distribution of these defects among children. There is dearth of information on the factors associated with developmental enamel defects; very few studies reported an association between fluoride levels in water and dental fluorosis. Several researchers in Nigeria have mentioned the need for further epidemiological studies that will examine the influence of other factors such as childhood illnesses, tooth cleaning practices, nutrition and genetics in the occurrence of developmental defects of enamel.

Chapter 2 Literature Review.

2.1 Introduction and methodology used

The aim of this literature review is to critically appraise the knowledge and ideas that have been established about the occurrence of developmental defects of enamel and dental fluorosis and factors associated with these defects with a view to undertaking a study amongst Nigerian children.

Keywords (Appendix A) focusing on key elements of the study aims such as developmental defects of enamel, dental fluorosis, dental caries, fluoride (F) intake, F excretion, indices for DDE and dental fluorosis, genetics, childhood illnesses, tooth cleaning practices, diets and children were developed. A detailed search strategy (Appendix B) was developed and used in Medline and then adapted to other databases (Scopus, Embase and Web of Science) using the keywords to identify studies and articles. This search strategy took into account the differences in controlled vocabulary and syntax rules for each database. All the results of the search were exported and compiled into Endnote version X7 (Thomas Reuters, 2013) and duplicate references removed. The titles and abstracts of the retrieved references were screened for relevance, irrelevant ones were removed and the remainder managed by grouping them based on the essential elements and categories of the study. Abstracts and then full papers of the relevant references were read and cited using cite-while-you-write-function in Microsoft word.

2.2 Morphological formation and maturation of the teeth

2.2.1 Formation of the teeth

An understanding of the morphological formation, maturation and development of the teeth is of significance in explaining the occurrence of developmental defects of enamel and dental fluorosis. Evidence of development of human teeth can be seen in the sixth week of embryonic life (Avery, 1987, Zhang et al., 2005). Teeth development or odontogenesis is a complex process by which embryonic cells derived from ectoderm of the first branchial arch and the ectomesenchyme of the neural crest aggregate as a tooth germ to form teeth which grow and later erupt into the oral cavity (Thesleff et al., 1995, Nanci, 2012). Tooth organogenesis is similar in all vertebrates which might explain why most mammalian tooth development research is undertaken on the mouse (Fraser et al., 2013, Jussila et al., 2014). The cells in the tooth germ proliferate and organize into three parts, namely, the enamel organ or (primary dental lamina), the dental papilla and the dental sac (or follicle) (Didilescu et al., 2013). The primary dental lamina, a horse-shoe shaped epithelial thickening in the embryonic jaws of mammals, proliferates and buds into the underlying neural crest

derived mesenchyme resulting in the condensation of mesenchymal cells around the epithelial bud (Jussila et al., 2014). These condensed mesenchymal cells then induce the formation of the primary enamel knot, an epithelial signalling centre in the distal end of the epithelial bud. The primary epithelial enamel knot then drives tooth morphogenesis through the cap and bell stages of development.

During the initiation stage, the dental lamina connects the developing tooth bud to the epithelial layer of the oral cavity. The bud stage is characterised by epithelial cells proliferating into the ectomesenchyme of the jaw thereby forming a tooth bud without clear arrangement of cells (Volponi et al., 2010). The tooth bud grows around the ectomesenchymal aggregation in form of a cap and the cells arrange themselves during the cap stage in preparation for histodifferentiation and morphodifferentiation. Histodifferentiation and morphodifferentiation take place during the bell stage and they begin with epithelial cells in contact with the dental papilla mesenchyme differentiating into ameloblasts and the adjacent dental papilla mesenchymal cells differentiating into odontoblasts (Thesleff et al., 1995). Ameloblasts are specialised cells that form enamel and they have distinct morphological characteristics that change at each developmental stage (Zheng et al., 2013). Generally, enamel formation occurs in two distinct phases, namely: the secretory and maturation phases. In the secretory phase, ameloblasts secrete enamel proteins such as amelogenins, ameloblastins, enamelins and tuftelins outwards away from the centre of the tooth thereby forming enamel matrix which is then mineralized, a process catalysed by the enzyme alkaline phosphatase (Nanci, 2012).

2.2.2 Maturation of the teeth

In the maturation stage, ameloblasts resorb enamel organic substances such as proteins and replace them with hydroxyapatite crystals. Secretory and maturation ameloblasts are characterised by expression of enamel stage-specific genes that perform stage-specific functions (Zheng et al., 2013). Therefore, an extensive series of reciprocal interactions between dental epithelium and the neural crest-derived mesenchyme results in the initiation, morphogenesis and cytodifferentiation of a tooth (Rothova et al., 2011, Nakamura and Fukumoto, 2013). After morphodifferentiation, the apposition and calcification of the enamel and dentine matrices follows. During this stage, a layer of an acellular extracellular matrix is deposited along the future amelodentinal and dentinocemental junction by ameloblasts and odontoblasts. After the laying down of the organic matrix, calcification begins with the precipitation and deposition of several small nidi of inorganic calcium salts which later coalesce. The mesenchymal cells within the dental papilla form the pulp of the teeth. The dental sac or follicle

give rise to the cementoblasts which form the cementum of the teeth, osteoblasts which form the alveolar bone around the roots and the fibroblasts which form the periodontal ligament that connect the cementum to the alveolar bone.

2.2.3 Molecular mechanisms in tooth development

Genes and genetic regulatory networks such as signalling pathways, transcriptor factors and various modulators are molecular mechanisms that play important roles in tooth organogenesis (Thesleff and Sharpe, 1997, Tummers and Thesleff, 2009, Jussila and Thesleff, 2012). Emerging evidence suggests that clock genes, a family of genes that control circadian functions within our bodies, also regulate dental mineralized tissues (Simmer et al., 2010, Lacruz et al., 2012, Sehic et al., 2013, Zheng et al., 2013). Enamel formation, for example, is influenced by short periods of rhythmical molecular signals that control the secretion and maturation of the enamel matrix (Zheng et al., 2014). The Bmp, Fgf, Shh and Wnt signalling pathways are repeatedly needed for tooth initiation and morphogenesis and are regulated by a number of activators and inhibitors acting in a highly integrated network (Lan et al., 2014). Other signalling pathways such as ectodysplastin (Eda), Sostdc and Lrp regulate tooth number, size and shape (Lan et al., 2014).

2.2.4 Relevance of chronology of tooth development

Human teeth start to develop during foetal development. Prenatally, primary teeth start to form between the sixth and eighth week *in-utero* while permanent teeth begin to form in the twentieth week *in-utero* lingual to the primary teeth germ. As Table 2.1 shows, the first evidence of calcification of primary dentition can be as early as 3 to 4 months *in-utero* and for the permanent dentition it can be as early as 3-4 months post-natally (Logan and Kronfeld, 1933). The differences in the timings of the chronological development of teeth are relevant when interpreting the pattern of distribution of developmental defects of enamel and dental fluorosis in different teeth and dentitions; the subject of this thesis. Disturbances in enamel occur when some elements e.g. F or drugs such as tetracycline and antimitotic drugs are administered at or above threshold during amelogenesis (Elfrink et al., 2013a, Lippert et al., 2014). Although F is essential in the prevention of dental caries, excessive F ingestion during amelogenesis can result in dental fluorosis or “mottled enamel” (Leverett, 1986, Do and Spencer, 2007, Spencer and Do, 2008, Vora and Vora, 2013). Dental fluorosis is a developmental disturbance of dental enamel caused by chronic excessive ingestion of F during tooth development resulting in enamel with lower mineral content (hypomineralised) and greater porosity (Fejerskov et al., 1990, Akpata et al., 1997).

Tooth	Age when calcification begins		Age when crown complete		Age at eruption	
Primary dentition	**Maxillary**	**Mandibular**	**Maxillary**	**Mandibular**	**Maxillary**	**Mandibular**
Central incisor	3-4 mo. i. u.	4.5 mo. i. u.	4 mo.	4 mo.	7.5 mo.	6.5 mo.
Lateral incisor	4.5 mo. i. u.	4.5 mo. i. u.	5 mo.	4.5 mo	8 mo.	7 mo.
Canine	5.5 mo. i. u.	5 mo. i. u.	9 mo.	9 mo.	16-20 mo	16-20 mo.
First molar	5 mo. i. u.	5 mo. i. u.	9 mo.	6 mo.	12-16 mo.	12-16 mo.
Second molar	6 mo. i. u.	6 mo. i. u.	10-12 mo.	10-12 mo.	20-30 mo.	20-30 mo.
Permanent dentition						
Central incisor	3-4 mo.	3-4 mo	4-5 y.	4-5 y.	7-8 y.	6-7 y.
Lateral incisor	10 mo.	3-4 mo	4-5 y.	4-5 y.	8-9 y.	7-8 y.
Canine	4-5 mo.	4-5 mo	6-7 y.	6-7 y.	11-12 y.	9-10 y.
First premolar	1.5-1.8 mo.	1.8-2 y.	5-6 y.	5-6 y.	10-11 y.	10-12 y.
Second premolar	2-2.3 mo.	2.3-2.5 y.	6-7 y.	6-7 y.	10-12 y.	11-12 y.
First molar	At birth	At birth	2.5-3 y.	2.5-3 y.	6-7 y.	6-7 y.
Second molar	2.5-3 y.	2.5-3 y.	7-8 y.	7-8 y.	12-13 y.	11-13 y.
Third molar	7-9 y.	8-10 y.	12-16 y.	12-16 y.	17-21 y.	17-21 y.

Table 2-1: Calcification, crown completion and eruption ages for primary and permanent teeth.

Source: Logan and Kronfeld (1933).

In summary, prenatally and post-natally, an extensive series of mutual interactions between cells in the tooth germ and neural crest-derived mesenchyme result in the initiation, histodifferentiation, morphodifferentiation and cytodifferentiation of a tooth under the regulation of genes and genetic pathways. Therefore, environmental and genetic interferences can result in enamel defects during tooth development. Further research among different population groups is needed to provide information on the effect of these disturbances on tooth development.

2.3 Defects in enamel development: aetiology and mechanisms of occurrence

2.3.1 Aetiologies of defects in enamel development

Enamel is a calcified tissue that contains a highly organized arrangement of inorganic crystals mainly hydroxyapatite (Margolis et al., 2006, Reyes-Gasga et al., 2012). Normal enamel includes a range of minor deviations in structure such as bending of enamel prisms and only variations outside this range can be considered pathological (Simmelink and Nygaard, 1979). Disturbances in the formation of enamel matrix, decreased availability of minerals for the calcification of the matrix and the incorporation of foreign materials into the matrix from genetic and environmental insults can alter the characteristics of enamel thereby affecting its colour, texture or thickness (Nanci, 2012). Enamel defects can be qualitative or quantitative in nature and can present with a wide range of clinical appearances (Elcock et al., 2006). Disturbances in enamel development can occur when there are changes in the number, quality and arrangement of the crystals during odontogenesis. Enamel defects can also be defined as aberrations in the quality and quantity of dental enamel caused by disruption and/or damage to the enamel organ (Salanitri and Seow, 2013).

Clinically they appear as visible deviations from the normal translucence of dental enamel resulting from enamel organ dysfunction (Suckling, 1989). The severity of an enamel defect depends on the stage of development during which the disturbance or insult occurs, as well as its extent and duration (Seow, 1991).

Enamel formation may be affected by hereditary, acquired, systemic and/or local factors and the changes induced in enamel can provide clues to determine the timing and nature of these events. Insults occurring during the earliest stages of enamel development i.e. matrix formation, will result in reduction in the amount or in the thickness of enamel (enamel hypoplasia) whereas insults occurring during the calcification and maturation stages of enamel development may lead to deficiency of mineralization (hypomineralization) and usually manifest as changes in translucency (enamel opacities) (Salanitri and Seow, 2013). These conditions are caused by systemic disturbances and

often involve tissues such as the skin that share common embryonic origins of neuroectodermal mesenchyme with teeth (Freiman et al., 2009). Factors impacting prenatally, perinatally or postnatally can cause these disturbances resulting in enamel defects. These factors include nutritional deficiencies such as protein and vitamins, febrile episodes from infections, chemical intoxications e.g. F, tetracyclines and metabolic conditions such as endocrinopathies (Freiman et al., 2009). Other factors reported in a review of developmental defects primary teeth include: ingestion of drugs such as thalidomides; prematurity/low birth weight, neonatal hypocalcemia, deprivation of sunlight, hyperbilirubinemia, thyroid and parathyroid disturbances, neonatal asphyxia; certain viral infections and genetic disorders such as amelogenesis imperfecta and tuberous sclerosis (Bhat and Nelson, 1989). Enamel defects has been associated with trauma and radiation and absence of breastfeeding (Seow, 1991, de Moraes Ramos-Perez et al., 2014). A cross-sectional study among older children - 80 Brazilian children aged 5-10-years showed that trauma, diseases, hygiene habits and F exposure were not associated with enamel defects (Cruvinel et al., 2012). In addition, a relationship has been suggested between X-Linked Hypophosphataemia (Cremonesi et al., 2014), frequent use of antibiotics such as penicillins and amoxicillin (Hong et al., 2011, Tariq et al., 2014), surgical closure of cleft palate (Carpentier et al., 2014), *in-vitro* fertilization (Kar et al., 2014) and enamel defects. A recent study of factors associated with molar incisor hypomineralisation (MIH) in Thai children suggested that caesarean section, complications during vaginal delivery and poor health during the first 3 years of life are independent risk factors for MIH but there was no association between preterm birth or low birth weight and MIH (Pitiphat et al., 2014). Enamel abnormalities occur from congenital abnormalities involving mineralisation pathways such as parathyroid disorders (McCauley and Martin, 2012). During amelogenesis, if there is hypocalcemia due to lack of parathyroid hormone which acts to regulate the balance of calcium, enamel defects could occur.

Furthermore, the role of systemic factors in the occurrence of enamel and skeletal defects was reported in a study on prevalence and severity of dental fluorosis and genu valgum (skeletal fluorosis) among school children in rural communities in India. In this study, dental fluorosis was positively associated with the occurrence of genu valgum, a skeletal form of fluorosis (Arvind et al., 2012). Enamel defects can be the result of variations in gene encoding for specific enamel proteins most directly involved in enamel bio-mineralization (Sheoran et al., 2014), or can occur as a feature of generalised familial conditions, i.e. inherited conditions (Freiman et al., 2009).

Regarding dental fluorosis, mild fluorosis appears as white flecks due to superficial porosity while more severe forms display staining, pitting and/or loss of enamel (de Carvalho et al., 2013). The classical appearance macroscopically is characterized by whitish bands that follow the development lines of the enamel and by symmetry on homologous teeth (Fejerskov et al., 1990). This disturbance caused by excessive systemic exposure to F occurs during late secretory and early maturation phases of enamel formation (Leverett, 1986). Dental fluorosis can occur when individuals are exposed to high concentrations of F in water, food, toothpaste, salt, condiments and other media and especially when multiple sources of systemic F are used in same individual (Rugg-Gunn et al., 1997, Rock and Sabieha, 1997, Lalumandier and Rozier, 1998). Early studies (Yadav et al., 2009, Mandinic et al., 2009) have shown that the prevalence and severity of fluorosis in a population is directly related to the concentration of F in drinking water in areas of naturally fluoridated water with concentrations over 0.7 ppm in hot climates producing greatly increased risk (Akpata et al., 2009). Dental fluorosis can also occur if F exposure persists over time, even at low concentrations, since it has a tendency to accumulate in individuals as a body burden (Arvind et al., 2012) and therefore, along with the time period of risk for dental fluorosis development, the amount of F intake must also be considered (de Carvalho et al., 2013).

F ingestion per kilogram body weight tends to increase with age in children less than 10 years of age (Akpata, 2004a), therefore teeth that mineralise later in a child's life would be liable to more severe fluorosis and this is in agreement with reported distribution of dental fluorosis by tooth type (Fejerskov et al., 1990). Excessive F intake can cause serious health problems if it affects skeletal tissue and soft tissues such as liver, kidney, brain, pancreas and thyroid glands (Xiong et al., 2007, Bashir et al., 2013). Children below the age of 8-years whose teeth and bones are still developing are most susceptible to all forms of fluorosis (Wambu et al., 2014). Dental fluorosis has also been shown to co-exist with iodine deficiency disorders (IDD), goitre, cretinism, low IQ, deaf mutism, knock-knee and bow legs in a comparative study (Singh et al., 2014) of F ingestion levels, serum thyroid hormone and thyroid stimulating hormone level derangements and dental fluorosis status among school children from endemic and non-endemic fluorosis areas of India. When testing drinking water for F content in endemic fluorosis areas, measurement of thyroid hormones in blood is helpful for recognising underlying thyroid derangements and their potential impact on fluorosis (Singh et al., 2014).

2.3.2 Developmental enamel defects and nutritional status/failure to thrive

The developing tooth germ is sensitive to a range of systemic disturbances causing irreversible damage therefore the tooth provides a repository of information on the timing and nature of insults potentially affecting other ectodermally derived structures including the brain (Levine et al., 1979). Developmental enamel defects could be useful historical markers for timing of intra-uterine or perinatal events associated with certain neurologic and sensory disorders of children (Bhat and Nelson, 1989). Prenatally originating defects can be caused by calcium deficiency in mothers who are malnourished or who avoid dairy products due to lactose intolerance while postnatally originating defects are often due to hypocalcemia in the infant resulting from insufficient calcium consumption or gastrointestinal malabsorption (Bhat and Nelson, 1989). Malnutrition and infections causing diarrhoea are two important factors implicated in the aetiology of hypoplasia in developing countries (Seow, 1991). The diarrhoea may have acted through induction of malabsorption of fat-soluble vitamin D and consequent failure to absorb calcium (Seow, 1991). Severe general under-nutrition as well as deficiencies in Vitamins A and D and of calcium can result in enamel defects (Seow, 1991).

Developmental enamel defects occur from prematurity, low birth weight and intra uterine growth retardation. Development enamel defects in the teeth of low birth weight children is caused by prenatal or postnatal alteration in calcium homeostasis and/or through endotracheal intubation and mechanical ventilation during postnatal period (Seow et al., 1989). The prevalence of enamel defects was reported to be high among a cross-section of premature and low birth weight children in Saudi Arabia (Rugg-Gunn et al., 1998), Brazil (Lunardelli and Peres, 2006), China (Li et al., 2006) and Tanzania (Masumo et al., 2013). Children who did not breastfed or who were breastfed for a shorter period had a higher prevalence of enamel defects than their counterparts (Vignarajah and Williams, 1992, Lunardelli and Peres, 2006).

2.3.3 Mechanism of occurrence of developmental enamel defects

Ultra-structural study of F-induced *in-vitro* hypermineralization of enamel of hamster tooth germs showed that excessive F causes secretory ameloblasts to change their rate of enamel protein synthesis or composition of secretory proteins (Lyaruu et al., 1986, Lyaruu et al., 2012). Water and secretory proteins such as amelogenins are retained resulting in enamel sub-surface porosity (DenBesten and Thariani, 1992). Bronckers et al. (1984) in their *in-vitro* study on effects of F on enamel and dentine formation in hamster tooth-germs in

organ culture suggested that, although the total amounts of amelogenins produced by the secretory ameloblast were not affected by F, an inhibition of amelogenin secretion by the cell occurred. In its distal cytoplasm, the secretory ameloblast exposed to excessive F showed accumulations of black globules and large clear vacuoles (Takuma et al., 1983), as well as accumulation of transport vesicles, disorganization of golgi apparatus and accumulation of abnormal large granules indicative of aberrant intracellular transport (Matsuo et al., 1996, Matsuo et al., 1998). Sufficiently large doses of F and tetracycline cause dilation of the rough endolasmic reticulum, reduction in the golgi apparatus and paucity of secretory granules thereby causing reduction in protein synthesis (Simmelink and Nygaard, 1979). A recent study on a potential molecular mechanism of enamel fluorosis in mice reported that F stimulates hypermineralization at the mineralization front, increasing proton release causing ameloblasts to secrete more bicarbonates which impede diffusion of proteins and mineral ions into the subsurface layers. These then delay bio-mineralization, causing retention of enamel matrix proteins (Lyaruu et al., 2014). F also affects the dentine where it increases interglobular dentine formation and accentuation of the incremental lines of von Ebner (Fejerskov et al., 1979). F at 50ppm can change the ratio of secreted amino acids (Patterson et al., 1976) by suppressing one of the protein species in developing enamel matrix resulting in fluorosed enamel (Simmelink and Nygaard, 1979). The effects of F and tetracycline on bone and teeth depend on their concentration in the blood serum (Focak et al., 2012) and both can form complexes with calcium in the blood (Lippert et al., 2014, Madhumathi and Sampath Kumar, 2014). Evans and Darvell (1995) hypothesized that early exposure to fluorosis-causing concentrates of F during the critical development period of 15-24 months makes ameloblasts more susceptible to a dose-dependent F challenge later during enamel maturation.

The aetiologies of developmental enamel defects are mainly from genetic and environmental insults. Prenatally, perinatally and postnatally, these insults can result in defects ranging from a reduction in the amount of enamel matrix (enamel hypoplasia) to a decrease in availability of minerals for calcification of the matrix manifesting as changes in translucency (enamel opacities). Globally, there is a need for more studies to explore the role of genetic and other influential environmental factors in the occurrence of developmental enamel defects.

2.4 Types of defects of enamel development: their appearance and consequences

Various terminologies and definitions are used to describe enamel defects (Clarkson and O'Mullane, 1989). Developmental defects of enamel (DDE) are visible deviations from the normal appearance of tooth enamel caused by enamel organ dysfunction (Chen et al., 2013). Some researchers (Mascarenhas, 2000, MacHiulskiene et al., 2009, Alvarez et al., 2009) have classified enamel defects into: a) F-induced (e.g. dental fluorosis) and non-F-induced (e.g. Developmental Defects of enamel (DDE) (Mohamed et al., 2010) while others (Vargas-Ferreira and Ardenghi, 2011, Chen et al., 2013) have classified them into quantitative (e.g. hypoplasia) or qualitative (e.g. opacity) defects. Generally, developmental defects of enamel are classified into three types, based on clinical appearance; hypoplasia, demarcated opacities and diffuse opacities including dental fluorosis (1992a, Targino et al., 2011). Other defects such as enamel opacities, tetracycline stains and enamel mutilation are among the more commonly seen anomalies of enamel formation (WHO, 1997).

2.4.1 Hypoplasia

Hypoplasia is a quantitative defect caused by incomplete deposition of immature enamel produced by ameloblasts during the secretory stage of tooth development resulting in reduced enamel thickness presenting as pits, grooves, thin and missing enamel (Jacobsen et al. 2014; Seow, 2014).

2.4.2 Demarcated opacities

Demarcated opacities or hypomineralization defects are qualitative defects that occur as a result of incomplete mineralization presenting as softened enamel (Clarkson and O'Mullane, 1992). Demarcated opacities can also occur as a result of reduced maturation of the enamel following the secretory stage of development resulting in alteration in the translucency of enamel (FDI, 1992b, Altun et al., 2009). In the primary dentition, the area of enamel affected by hypomineralization has lower mineral content compared to sound enamel (Costa-Silva et al., 2013).

2.4.3 Diffuse opacities (including dental fluorosis)

Diffuse opacities of enamel are the feature commonly used to distinguish the teeth of children in fluoridated and non-fluoridated areas however, not all diffuse opacities are caused by excessive exposure to F (Suckling and Pearce, 1984, Sabieha and Rock, 1998). Dental fluorosis, especially in its mild form, is a diffuse opacity due to hypomineralisation, while in a severe form it is due to the low level of mineralisation of the enamel resulting in

a characteristic appearance in terms of tooth appearance and distribution in the mouth (Mabelya et al., 1992). Fluorotic enamel has an altered structure and appearance that becomes more severe as the duration and amount of F absorption increases (Den Besten, 1994, Fejerskov et al., 1994). The severity of dental fluorosis is directly related to the amount of F in the enamel and the amount of subsurface porosity (Fejerskov et al., 1994, DenBesten, 1999). The severity is also related to increasing age which suggests that prevalence rate increases with increase in duration of exposure to F during the period of tooth formation (Srivastav et al., 2011, Arvind et al., 2012). The clinical appearance of dental fluorosis is characterised by bilateral opaque white areas in enamel which become striated, mottled and/or pitted with increasing levels of F ingestion (DenBesten, 1999). The mildest form of fluorosis is characterized by opaque white lines due to accentuated perikymata and in some cases the white lines may be confluent while in others there may be discrete white opaque areas. However, in severe cases the entire enamel surface is chalky white and the hypoplasia may lead to confluent pitting with associated loss of normal tooth form (WHO, 1997). The opaque areas may become stained yellow or dark brown in severe fluorosis (Sundström et al., 1980, Fejerskov et al., 1991). Tooth discoloration occurs when the sub-surface porosity attracts extrinsic stains post-eruptively (Fejerskov et al., 1994). Additionally, post-eruptive trauma causes detachment and pitting of enamel surface when there is extensive sub-surface porosity. The enamel discoloration and pitting can be aesthetically objectionable and has been found to be a cause of psychological ill health in a study of altitude as a risk indicator of dental fluorosis in children residing in areas with 0.5 and 2.5 mg F in drinking water (Rwenyonyi et al., 1999).

Histopathologically, non-pitted fluorotic enamel shows a subsurface porosity below a well mineralized surface zone (Triller, 1979). The whiter more opaque appearance of the enamel is a result of the subsurface porosity and with increasingly severe fluorosis, the porosity extends toward the dentinal-enamel junction and the enamel surface can break down after eruption resulting in pitting of enamel (Fejerskov et al., 1991).

Developmental enamel defects are clinically significant since they are responsible for aesthetic problems, dental sensitivity, erosion, wear, dentofacial anomalies as well as predisposition to dental caries (Aine et al., 2000, Contaldo et al., 2014).

The terms commonly used to describe enamel defects are defined in Table 2.2.

Term	Definition
Opacity	Altered translucency
Diffuse opacity	Opacity distributed over a relatively large area
Demarcated opacity	Opacity confined to a relatively small area
Hypoplasia	Reduction in quantity of tissue formed
Hypomineralization/hypocalcification	Reduction in deposition of mineral
Hypomaturation	Reduction in the deposition of mineral during the maturation stage of mineralization
Fluorosis	Hypomineralized and faint white flecks or opaque white or paper white enamel which becomes striated, mottled, stained yellow or dark brown and/or pitted

Table 2-2: Terms and definitions applied to defects of enamel development.

Source: Seow (2014).

The enamel defects do not directly increase the risk of occurrence of dental caries in the affected teeth but the absence of normal enamel morphology invariably results in reduced occlusal function (Brindha and Elango, 2011). In the development of self-esteem in children, an aesthetically pleasant appearance of newly erupted anterior teeth is of great importance (Brindha and Elango, 2011).

The classification and terms commonly used to describe developmental enamel defects have helped to develop indices use for measuring these defects directly or indirectly in epidemiological surveys among various population groups.

2.5 Measurement of developmental enamel defects including fluorosis

2.5.1 Indices for measuring developmental enamel defects

The use of different classifications and indices for measuring developmental enamel defects has complicated direct comparison of the findings of population surveys of defects of enamel development (Mohamed et al., 2010). Correa-Faria and co-workers (Corrêa-Faria et al., 2014), in their cross-sectional study on the association between DDE and Early Childhood Caries (ECC) reported that, when studies were compared, it was difficult to establish a consensus on this association due to the different assessment tools employed for the diagnosis of DDE. The choice of index can influence the investigation of developmental enamel defects (Corrêa-Faria et al., 2014) while the accurate recording of developmental defects is important for clinical, diagnostic, medico-legal purposes and aetiological studies. The indices which have been used to measure developmental enamel defects can be categorised into: a) general indices such as the Young (Young, 1973), Al-Alousi et al (Al

Alousi et al., 1975), Developmental Defects of Enamel (DDE) (FDI, 1982) and modified Developmental Defects of Enamel (mDDE) (Clarkson and O'Mullane, 1989) indices which classify the full range of enamel defects in a descriptive way and not based on their aetiology. b) specific indices such as Dean (Dean, 1934), Thylstrup and Fejerskov (Thylstrup and Fejerskov, 1978) and Tooth Surface Index of Fluorosis (Horowitz, 1986) indices which only identify enamel defects due to excessive F ingestion. These two categories of index have a fundamental difference when measuring defects due to F, even when used in the same population. The suitability of an index used for a study will depend on the aims of that study.

2.5.1.1 Developmental Defects of Enamel index

A diagnosis of dental fluorosis is difficult to make and accept, especially in low-F areas, since it often confuses examiners and is not based on descriptive criteria. Descriptive indices are simple to use and examiners do not need to pay attention to distinguishing between F-related opacities and non-F-related opacities. Descriptive indices such as Young (Young, 1973) and Al-Alousi et al (Al Alousi et al., 1975) indices were proposed but they failed to unify the numerous classifications of enamel opacities as they also used additional terminology thereby causing confusion. A Working Group of the FDI devised the general descriptive index entitled Developmental Defects of Enamel (DDE) Index in order to formulate a well-defined classification which fulfilled the general requirement for measuring enamel defects (FDI, 1982). The index was later modified in 1992 (FDI, 1992b) and is recommended by the World Health Organization (WHO, 1997) for use in oral health surveys. This modified Developmental Defects of Enamel (mDDE) index has a simple classification and uses standard terminology which is more effective for measuring the more important types of enamel defects including the colour, demarcation and hypoplastic defects (Clarkson and O'Mullane, 1989). The DDE index records a broad array of defects with no aetiological assumption thereby defects are classified into demarcated opacities, diffuse opacities or hypoplasia or combinations of these (Mohamed et al., 2010). The descriptive nature of the DDE index allows determination of the overall prevalence of developmental enamel defects since it records both F and non-F induced defects. Therefore the DDE index may be more appropriate than a fluorosis-specific index for measuring any kind of enamel defect. However, the DDE index is relatively complex and time-consuming to use especially when a number of defects are present.

2.5.1.2 Dean's index

Dean's index, one of the most commonly used dental fluorosis indices, measures the severity of enamel defects caused by excessive F ingestion by the allocation of a score to the examinee based on the two most severely affected teeth. If the two teeth are not equally affected, the score of the less affected tooth is recorded. Changes in enamel are assigned scores ranging from 0 (normal) to 5. This index has been criticised because it does not give sufficient information on the distribution of dental fluorosis within the dentition since each individual receives a mouth score corresponding to the clinical appearance of the second most severely affected tooth in the mouth and thereby does not record isolated defects (Horowitz, 1986, Clarkson, 1989). In addition, Dean's score data are ordinal, not continuous and using the scale involves averaging the scores which is inappropriate (Mohamed et al., 2010). Despite these criticisms, Dean's index remains a popular index, particularly in the United States (McGrady et al., 2012b), and continues to be widely used, especially for historical comparisons. From this index, (Dean, 1942) also developed the Community Fluorosis Index (CFI) to compare one population group with another on the basis of average severity of fluorosis.

2.5.1.3 Thystrup and Fejerskov index (TFI)

Thylstrup and Fejerskov (1978) proposed an index to measure enamel defects of F origin based on their histological appearance. They suggested that the detailed histologically derived surface classification was more sensitive and reliable in order to establish a better understanding of the relationship between an individual's exposure to F during the period of tooth formation and dental fluorosis. Changes in enamel are arranged into 10 classes that are assigned scores ranging from 0 (normal) to 9 with each score representing measurement on an ordinal scale. Every tooth surface – buccal, occlusal and lingual - is dried prior to examination which creates an unnatural appearance and makes minor defects appear more clearly. These surfaces may have their own score.

2.5.1.4 Tooth Surface Index of Fluorosis (TSIF)

The National Institute of Dental Research in the United States developed the TSIF to estimate the amount of fluorosis as a fraction of the total visible enamel surface (Horowitz et al., 1984). This index records the presence of fluorosis on a tooth- and a tooth-surface basis and using an ordinal scale. Only permanent and unrestored teeth are scored, with a separate score for each tooth surface; two scores are assigned to anterior teeth and three to

posterior teeth. Changes in enamel are arranged into 8 classes that are assigned scores ranging from 0 (normal) to 7.

2.5.1.5 Comparing indices use for measuring developmental enamel defects

Dean's index assesses teeth wetted by saliva while the TF index requires the drying of teeth prior to assessment, highlighting the presence of more mild presentations of fluorosis (McGrady et al., 2012b). A study on analysis of three dental fluorosis indices in epidemiologic trials among 461 school children aged 12-14-years living in 3 Brazilian cities showed similar percentages of children are affected by dental fluorosis in the 3 cities when the 3 indices (Dean, TF and TSIF indices) were used to assess the defects. There were no difficulties in using the three indices in the field trials therefore, the use of any of them was strongly recommended (Pereira and Moreira, 1999). A study that compared Dean's and DDE indices in examining dental fluorosis and diffuse opacities among 9-year-old children found relatively little concordance between the 2 indices in determining the prevalence of these defects among these children (Mohamed et al., 2010). However, this study did indicate that at tooth level, concordance was greater, suggesting that little may be lost in fluorosis studies which use the DDE index, particularly as it enables collection of a wider and more comprehensive range of information on defects. Similarly, studies that assessed enamel defects using the TF and modified DDE indices concluded that there was good agreement between the 2 indices (Sabieha and Rock, 1998, Khan, 2005). Khan (2005) concluded that if all diffuse defects recorded using the mDDE index were assumed to be fluorosis as a result of F intake then fluorosis may be over-estimated compared with fluorosis recognized by the Thystrup and Fejerskov index.

2.5.2 Methods of measurement

Developmental enamel defects can be detected and assessed using macroscopic and microscopic methods or both. Macroscopic methods such as direct clinical observations employing direct visual and tactile examinations and photographic methods are valuable in epidemiological studies. Microscopic methods such as polarised light microscopy, scanning electron microscopy (SEM), reflectance confocal microscopy (RCM) and optical techniques have also been used to measure developmental enamel defects but are not a primary approach for community-based dental epidemiology.

2.5.2.1 Macroscopic method: Direct clinical method

The most generally accepted macroscopic method of measuring developmental enamel defects is the direct clinical examination and this might be due to it being quick, of low cost

and providing the ability to examine all surfaces of teeth (Golkari et al., 2011). Direct clinical examination can be undertaken under natural light but direct sunshine should be avoided (Clarkson and O'Mullane, 1989). A fibre optic light can be used when natural light is not strong enough, or when posterior teeth are being examined (Golkari et al., 2011). Prior to the examination, the teeth may be cleaned so that their surfaces are devoid of debris that may affect the assessment (Evans, 1991). A number of concerns and limitations of using direct clinical examination have been expressed by researchers. For example, it relies upon subjective evaluation and interpretation of predetermined criteria which permits the introduction of unintentional observer bias and subjectivity especially when an underlying issue may be a sensitive and emotive one, as in water fluoridation (Dooland and Wylie, 1989, Levine et al., 1989). In addition, it can cause visual problems for the examiner (Golkari et al., 2011) and requires subject participation in the clinical examination for a considerable time period therefore can be difficult to use more than two different epidemiological indices (Sabieha and Rock, 1998). Furthermore, when clinical examinations are conducted by different examiners and at different times, there are concerns about the appropriateness of comparing the data (Nunn et al., 1993, Cochran et al., 2004a).

2.5.2.2 Macroscopic method: Indirect clinical method (Photography)

These concerns and limitations of direct methods of measurement have led to the use of photographic images to simulate clinical examination when assessing enamel defects. Photographic methods have also been employed to assist diagnosis alongside clinical examinations and increase the accuracy in detecting these defects (Cochran et al., 2004a). Photographic methods offer several advantages over clinical examination in terms of randomness, blinding, remote examinations, objectivity, permanent records for future comparisons, subject and examiner comfort and applications of different approaches in the utilization of the same materials (Nunn et al., 1992, Cochran et al., 2004c, Golkari et al., 2011). In addition, consensus scoring of remote images may address some of the issues relating to personal thresholding (McGrady et al., 2012b). On the other hand, there are disadvantages associated with the use of photography, namely cost and inability to use tactility. In addition, there are technical limitations to photographic assessment of enamel defects, for example, only the anterior teeth can be photographed easily and teeth such as lateral incisors and canines are rotated or overlapped in the photographic slide or film because they are at the sides of the field of view (Wong et al., 2005). To overcome this rotation and overlap, it has been suggested that multiple lateral views should be taken

(Ellwood and O'Mullane, 1995) but this adds to the complexity of the method. Further technical limitations of photographic assessment of enamel defects are burn-out caused by camera flash and reflections of light which obscure parts of a tooth surface when viewed on a photographic slide (Wong et al., 2005). To minimise burn-out, multiple photographs should be taken from positions superior and inferior to the horizontal plane at varying degrees (Fleming et al., 1989, Ellwood et al., 1996).

Studies that have used photographic methods as a means of assessing developmental enamel defects have demonstrated a high reliability in their use for assessing DDE (Wong et al., 2005, Elfrink et al., 2009, Golkari et al., 2011) with high intra and inter-examiner reliability (Wong et al., 2005, Elfrink et al., 2009) although one study on evaluating enamel opacities using standardized photographic method reported a relatively low inter-examiner reliability because the teeth were graded using both DDE and TF indices (Cochran et al., 2004c). A study on assessment of DDE using photographs concluded that multiple-view photographic slides of 'five-view' and 'three-view' are valid and reliable while 'one-view' was acceptable to assess only the incisors (Wong et al., 2005). Conventional photography was preferably used by some researchers to determine the presence of enamel defects (Kanthathas et al., 2005, Wong et al., 2005). However, better conditions to record developmental enamel defects are provided by digital photography (Wong et al., 2005) since images of the teeth can be viewed immediately and repeated if there is a problem such as burn-out. Additionally, digital photography allows many photos to be taken and those with fewer technical errors chosen (Bengel, 2006). Images of the teeth can be magnified resulting in increased detection of milder forms of dental fluorosis which may affect prevalence of enamel defects (McGrady et al., 2012b). Digital photography is cheap and does not depend on developing negatives as well as printing or projection (Golkari et al., 2011). All the limitations of using indices of measuring developmental enamel defects cannot be totally eliminated or controlled since the assessment of developmental enamel defects using digital imaging primarily relies on an examiner employing a subjective index (Golkari et al., 2011).

2.5.2.3 Microscopic methods

Microscopic methods such as polarised light microscopy, scanning electron microscopy (SEM), reflectance confocal microscopy (RCM) and optical techniques such as quantitative light induced fluorescence (QLF) have been used to study morphological appearance of enamel (Sabel et al., 2009, Huang et al., 2011, Umemoto et al., 2012, Pretty et al., 2012, Contaldo et al., 2014). For example, polarised light microscopy and scanning electron

microscopy were used to investigate enamel hypoplasia in exfoliated primary teeth and showed that cervical and incisal borders of enamel hypoplasia had a rounded appearance with a porous base constituting possible pathways for bacteria into dentine (Sabel et al., 2009). There are limitations to the use of QLF such as aberrant readings for dental fluorosis quantification due to its inability to distinguish between fluorescence loss from fluorosis and other defects such as enamel fractures and extrinsic stains (McGrady et al., 2012b). However, QLF still showed potential as a means of objective, blinded quantification and a means of providing a system for longitudinal monitoring of dental fluorosis (McGrady et al., 2012b). A cross-sectional study that evaluated the use of QLF for the quantification of dental fluorosis in a survey of 1774 UK children in fluoridated and non-fluoridated communities showed that a fluorescence imaging system when used together with photographic scoring may provide a useful objective, blinded system for the measurement of enamel fluorosis (McGrady et al., 2012b).

Replicas of the teeth may be used in both microscopic and macroscopic studies of developmental enamel defects (Golkari et al., 2011). A major disadvantage of this method of assessing enamel defects is that teeth are presented in one colour therefore changes in enamel colour are not shown, however, hypoplasia including small changes in the enamel surface can be better viewed microscopically or macroscopically (Golkari et al., 2011).

2.5.2.4 Comparison of direct and indirect methods

Some studies have compared the results of detecting developmental enamel defects using direct clinical examination, photographic and replica methods (Ellwood et al., 1996, Sabieha and Rock, 1998, Wong et al., 2005, Golkari et al., 2011, Chen et al., 2013). Wong and co-workers (Wong et al., 2005) used the mDDE index to compare direct clinical examination and photographic methods and showed Kappa agreement values of 0.79-0.85 between them for detecting subjects with any DDE. For the photographic method, the intra-examiner reliability was (k=0.81-0.88) while it was (k=0.82) for direct clinical examination. Ellwood and co-workers (Ellwood et al., 1996) also found agreement between clinical and imaging methods when the TF index was used with both methods. Similarly, Sabieha and Rock (1998) reported good agreement between the TF and modified DDE indices when recording the distribution of milder types of enamel in a population of 9-year-old children. However, a study (Golkari et al., 2011) that compared photographic, replication and direct clinical examination methods for detecting DDE showed that for epidemiological surveys the photographic method was the best of the three methods because it was more sensitive than a direct clinical examination. Similarly, in a cross-

sectional study (Cruz-Orcutt et al., 2012) that assessed and compared photographic and clinical examination of fluorosis using the fluorosis risk index (FRI) among 538 13-year-old children in the Iowa F Study (IFS), inter-examiner reliability was greater and fluorosis scores were higher when using photographic imaging. Photographic examination detected significantly more defects than clinical examination using DDE index, regardless of age group and type of defects, in a study (Chen et al., 2013) that evaluated agreement between photographic and clinical examinations among children and infants.

2.6 Epidemiology of Developmental Defects of Enamel (Table 2.3)

Epidemiological surveys have reported an increase in the prevalence of DDE in populations and this increase in the prevalence of DDE might be due to increase in the aetiological factors and better detection methods. (Seow et al., 2011). Information on the prevalence and pattern of distribution of developmental defects of enamel will help to identify adverse conditions that could affect the teeth during their development. This information will also help to provide oral health policies that will prevent and manage the occurrence of development enamel defects. The prevalence of DDE is commonly reported as mouth and tooth prevalence. Mouth prevalence is determined by the inclusion of any individual who has been found to have at least one tooth affected by the defect thereby demonstrating the extent of the distribution of enamel defects in a population group, however, individuals who are mildly and severely affected are grouped together using this tool (Anthonappa and King, 2015). Tooth prevalence shows the mean number or percentage of teeth affected per person and thereby indicates the proportion of teeth affected and hence provides some indication of the severity of the condition (Anthonappa and King, 2015).

Table 2.3 summarises the prevalence of DDE in both primary and permanent dentitions as reported in the literature based on regions, water F concentration, number of study participants, age, dentition, index used for measurements, examination conditions and results (mouth and tooth prevalence). Overall the majority of the studies were from South America and Asia and this might be due to the high prevalence of DDE reported in these regions. Sizeable numbers of studies have reported from other regions because these defects are of public health concern. The majority of the studies reported the mouth prevalence of DDE rather than the tooth prevalence because it is easier to collect and report mouth prevalence data. Generally, the reported prevalence of DDE can vary widely due to the use of various terminologies and the different diagnostic criteria and examination conditions employed to describe DDE defects (demarcated opacities, diffuse opacities and hypoplasia)

in both primary and permanent dentitions. For example, for all types of DDE, in the <0.2 ppmF water areas the published mouth prevalence in primary teeth ranged from 3.9% reported for 5 and 9-year-old American children (Hong et al., 2009) to 81.3% in 1 - 4.5 year-old Brazilian children (Targino et al., 2011). Similarly, the mouth prevalence of the different types of DDE in permanent teeth ranged from 9.8% among 11-13-year-old Italian children living in a water F area of 0.3 ppm (Angelillo et al., 1990) to 92.1% in 12-year-old Hong Kong children who lived in an area of 1.0 ppm water F (Wong et al., 2006). The differences in the prevalence of DDE in the permanent dentition in relation to F concentration of water in these two studies show a relationship between F concentration in water and the mouth prevalence of DDE; particularly diffuse opacities. This was also in agreement with findings in a study among 11 – 13 year-old Italian children (Angelillo et al., 1990) and 14 year-old Sri-Lankan children (Ekanayake and Van Der Hoek, 2003) where the prevalence of DDE increased as the F concentration of drinking water increased. Furthermore, a water defluoridation programme in Hong Kong showed that as the F concentration in water decreased from 1 ppmF in 1983 to 0.5 ppmF in 2001, the prevalence of DDE decreased from 92.1% to 35.2% in the respective years (Wong et al., 2006). No studies have reported a relationship between F concentration in water and the prevalence of DDE in primary teeth primarily because during the development of primary teeth *in utero* the placental barrier helps to protect the developing teeth from the effect of excessive F. In addition, after birth, children are exposed to breast milk which contains very low amounts of F and also helps to protect primary teeth from the effect of excessive F.

The majority of the studies used the mDDE as the index of measuring DDE because it classifies the full range of enamel defects in a descriptive way and it is recommended by WHO for use in oral health surveys. Of the many studies undertaken, two studies used the MIH criteria which is a specific index used to measures hypomineralization. These studies reported tooth prevalence of DDE in primary teeth reported 1.7%, 4.1% and 21.2% among 5 and 9-year-old US (Hong et al., 2009), 3-12-year-old Spanish (Robles et al., 2013) and 1-4-year-old Thai children (Kanchanakamol et al., 1996) respectively. Similarly, only 2 studies reported a tooth prevalence of DDE in permanent teeth ranging from 2.2 to 20.3% (Angelillo et al., 1990, Yusoff et al., 2008). Tooth prevalence of DDE can also be reported as mean number of teeth affected by defects.

Authors (date)	Country	Water F level (ppm)	N	Age (years)	Dentition	Index	Exam conditions & Notes	Results	
								Mouth Prev. (%)	Tooth Prev. (%)
Europe									
Dummer et al. (1986)	UK	<0.1	759	11 – 12	Permanent	DDE	Teeth dried but not cleaned under dental lighting. Most teeth affected equally on right and left sides. Maxillary incisors mostly affected. Defects mostly on buccal surfaces.	NR	8%-Max. 3.6%-Mand.
Dummer et al. (1990)	UK	<0.1	791	15 – 16	Permanent	DDE	Teeth dried but not cleaned under dental lighting. 7.6% - Maxillary.; 4.3% - Mandibular; Demarcated opacity (28.3%) while diffuse opacity (10.2%). Most teeth affected equally on right and left sides. Maxillary incisors mostly affected.	50.1	5.7
Angelillo et al. (1990)	Italy	0.3 1.0 4.0	643	11 – 13	Permanent	DDE	Demarcated opacity most common; Central incisors mostly affected in maxilla while first premolars and molars mostly affected in mandible.	9.8 23.0 53.1	2.2 5.7 20.3
Wogelius et al. (2008)	Denmark	0.05-0.73	745	6 – 8	Permanent	MIH criteria Weerheijm and Mejàre (2003)	Brushed and cleaned with cotton wool; Demarcated opacity most common and maxillary central incisors teeth mostly affected.	43.6	NR
Robles et al. (2013)	Spain	0.07	1414	3 - 12	Primary Permanent	mDDE	Dentist under artificial light, tooth brushed and dried. Diffuse opacity most common in primary while demarcated opacity most common in permanent teeth.	40.2 52	4.1 8.3
North America									
Slayton et al. (2001)	USA	NR	698	4 – 5	Primary	mDDE	Examination light without drying; No significant difference in the prevalence of enamel defects between boys and girls but significantly more boys than girls had enamel opacities.	6 – H 27 - O	0.13* 0.50*
Montero et al. (2003)	USA	NR	517	3 – 5	Primary	mDDE	Natural lighting; No significant difference in the prevalence of enamel defects among race; anterior teeth	49	NR

Authors (date)	Country	Water F level (ppm)	N	Age (years)	Dentition	Index	Exam conditions & Notes	Results	
								Mouth Prev. (%)	Tooth Prev. (%)
							had majority; an association between enamel defects and caries was observed.		
Hong et al. (2009)	USA	NR	491	5 & 9	Primary molars	mDDE	Examination light and teeth not dried; teeth with enamel hypoplasia had a significantly higher risk of dental caries.	3.9	1.7
South America									
Vignarajah and Williams (1992)	Antigua	NR	482	3 – 4	Primary	NR	Children with enamel defects breast-fed for a shorter period when compared to children without enamel defects who breast-fed for a longer period.	24	NR
Lunardelli and Peres (2005)	Brazil	NR	431	3-5	Primary	mDDE	Natural light without prior drying and dental prophylaxis; diffuse opacities most common defects; second molars were mostly affected and defects more common in upper arch.	24.4	NR
(Lunardelli and Peres, 2006)	Brazil	NR	102 113	3-5 DDE 3-5 No DDE	Primary	mDDE	Natural light without prior drying and dental prophylaxis; prematurity and lack of breastfeeding were associated with enamel defects.	NR	NR
Soviero et al. (2009)	Brazil	NR	292	7-13	Permanent	MIH criteria Weerheijm and Mejàre (2003)	Dentist using artificial light after brushing but no drying; demarcated opacity most common defect and 1st molars and upper central incisors were teeth mostly affected.	40.2	1.98*
Casanova-Rosado et al. (2011)	Mexico	NR	1296	6-12	Primary Permanent	mDDE	Natural light; for each primary tooth with DDE, the odds of observing DDE in the permanent dentition was increased 7.38 times.	10 7.5	NR
Targino et al. (2011)	Brazil	<0.2	275	1-4.5	Primary	mDDE	Natural light; teeth cleaned with gauze; enamel defects were associated with caries.	81.3	NR
Corrêa-Faria et al. (2013b)	Brazil	NR	381	3-5	Primary	DDE	Natural light; teeth cleaned with gauze; Demarcated opacity most frequent type of defect. Very low birth weight babies had a greater prevalence of enamels	29.9	NR

Authors (date)	Country	Water F level (ppm)	N	Age (years)	Dentition	Index	Exam conditions & Notes	Results	
								Mouth Prev. (%)	Tooth Prev. (%)
							defects. Prematurity and socio-demographic variables were not associated with enamel defects.		
Gravina et al. (2013)	Brazil	NR	96 96	Preterm Term	Primary	NR	A higher prevalence of enamel defects found in the premature group, with a predominance of hypoplasia; A significant correlation found between very low birth weight and the presence of defects (p < or = 0.001). The teeth most affected were incisors, canines and molars.	NR	NR
Vargas-Ferreira et al. (2014)	Brazil	NR	1210	8-10	Permanent	mDDE	Diffuse opacity most common defect; dental caries associated with enamel hypoplasia.	64	NR
Asia									
Li et al. (1995)	China	NR	1344	3-5	Primary	mDDE	Examination under natural light and teeth not dried or cleaned; hypoplasia most common defect; max central incisors and lateral incisors mostly affected by enamel hypoplasia.	23.9	NR
Kanchanakamol et al. (1996)	Thailand	NR	344	1-4	Primary	NR	Teeth cleaned with gauze; enamel hypoplasia most common defect; max. central incisors mostly affected than lateral incisors; caries associated more with enamel hypoplasia than opacities and sound enamel.	31.9	21.2
Ekanayake and Van Der Hoek (2003)	Sri Lanka	<0.3 0.31-0.49 0.5-0.7 >0.7	486	14	Permanent	mDDE	Maxillary first premolars mostly affected by diffuse opacities.	29 35 43 57	NR NR NR NR
Lo et al. (2003)	China	Non-fluoridated	250	12	Primary	mDDE	Higher prevalence of demarcated opacities was found in permanent teeth of which their primary predecessor had caries than in those without.	56.8 – DO 10.8 - H	5.8 1.3
Wong et al. (2006)	Hong Kong	1.0(1983) 0.7(1991)	1990	12	Permanent	mDDE	Intraoral radiographs taken and log time follow up study; diffuse opacities most common defects, marked differences in the mean number of teeth affected by	92.1 55.8 35.2	NR NR NR

Authors (date)	Country	Water F level (ppm)	N	Age (years)	Dentition	Index	Exam conditions & Notes	Results	
								Mouth Prev. (%)	Tooth Prev. (%)
		0.5(2001)					DDE and in the maximum extent of DDE between 1983, 1991 and 2001 were also observed.		
Yusoff et al. (2008)	Malaysia	NR	957	11-12	Permanent	mDDE	Malys have the highest prevalence while Chinese the least; diffuse opacities most common defects; most subjects showed bilaterally distribution of diffuse and posterior teeth commonly affected.	90.7	47.2
Lin et al. (2011)	China	NR	135	1.5-6	Primary	mDDE	Teeth cleaned with gauze and examined in wet condition. Enamel hypoplasia most common defects; most defects located symmetrically in primary incisors and first molars.	32.6	NR
Chauhan and Chauhan (2013)	India	NR	1188	9 & 12	Primary Permanent	mDDE	Teeth cleaned and dried when necessary. Diffuse opacity most common while enamel hypoplasia was least prevalent defect.	51.3	NR
Australasia									
Mackay and Thomson (2005)	New Zealand	NR	476	9 & 10	Permanent	mDDE	Teeth wet and uncleaned. Demarcated opacity most common; max central incisors were teeth commonly affected; diffuse opacity frequent among children who lived all their lives in fluoridated areas.	51.6	NR
Broadbent et al. (2005)	New Zealand	NR	663	5 & 9	Primary Permanent	mDDE	Where a primary tooth had been carious, the permanent successor was more likely to have a demarcated opacity after adjustment for gender, family socio-economic status, years of exposure to water fluoridation, trauma to primary teeth, and early loss of primary teeth	NR	NR
Arrow (2008)	Australia	0.8	511	7	Permanent first molars	mDDE	Dental lighting, tooth air dried, debris removed; Of the first permanent molars, 42 per cent upper right; 37 per cent upper left; 47 per cent lower left; and 45 per cent lower right were without enamel defects. White diffuse opacities were the predominant enamel defects	71	NR
Schluter et al. (2008)	New Zealand	FA NFA	612 310	9	Permanent	mDDE	Teeth undried, debris removed; diffuse opacity most prevalent enamel defect;	29.1 14.7	NR NR

Authors (date)	Country	Water F level (ppm)	N	Age (years)	Dentition	Index	Exam conditions & Notes	Results	
								Mouth Prev. (%)	Tooth Prev. (%)
Kanagaratnam et al. (2009)	New Zealand	FA NFA	612	9	Primary Permanent	mDDE	Teeth undried, debris removed; After adjustment for covariates, a strong dose-response relationship between diffuse opacity and fluoridation status and conversely, a strong protective dose-response relationship between caries experience and fluoridation status.	19 - Diffuse	NR
Seow et al. (2011)	Australia	0.1	517	NR	Primary Permanent	mDDE	In primary teeth enamel opacity common than enamel hypoplasia and demarcated opacity predominant compared to diffuse opacity while missing enamel most common type of enamel hypoplasia. In permanent teeth defects were more variable.	25 58	NR NR
Middle East									
Rugg-Gunn et al. (1997)	Saudi Arabia	0.22 0.78 2.66	1,539	14	Permanent	DDE	Diffuse defects most common. Multivariate analyses revealed that all three variables-region, nutritional status, socio-economic status-were statistically significantly related to the prevalence of defects and the number of teeth affected: prevalence was highest in the region with the highest water fluoride concentration, in rural areas and in malnourished subjects. Maxillary incisor teeth most affected teeth in all regions	83	9.6
Rugg-Gunn et al. (1998)	Saudi Arabia	0.22 0.78 2.66	390	2, 4 or 6	Primary	DDE	Malnutrition, low birth-weight, childhood illness, brushing of child's teeth and swallowing toothpaste were related to the prevalence of developmental defects of primary teeth	NR	NR
(Farsi, 2010)	Saudi Arabia	NR	510	4 & 5	Primary	mDDE	Demarcated opacities most prevalent defect, followed by hypoplasia. The most frequently affected teeth were maxillary anterior teeth, while the least affected teeth were mandibular incisors. A positive association between DDE and caries was observed.	45.4	NR
Memarpour et al. (2014)	Iran	0.3-0.7	974	9 & 11	Permanent	mDDE	Dental students undertook dental examination; teeth were cleaned; defects were significantly related with Apgar score at birth <7 and illness during the first	48.2	NR

Authors (date)	Country	Water F level (ppm)	N	Age (years)	Dentition	Index	Exam conditions & Notes	Results	
								Mouth Prev. (%)	Tooth Prev. (%)
							month. DDE showed no significant relationship with gestational age, delivery type, birth weight, gender or type of feeding during early infancy.		
Africa									
Sawyer et al. (1984)	Nigeria	NR	2203	10-19	Permanent	NR	Examination done under natural and/or artificial light.	11.7	NR
Koleoso (2004)	Nigeria	NR	200	12	Permanent	mDDE	Teeth examined wet; dental fluorosis was present in 36.5%, 21.5% had tetracycline stains, 16.0% had enamel opacities, 7.0% had enamel hypoplasia and 2.5% presented with enamel mutilation	42.5	NR
Orenuga and Odukoya (2010)	Nigeria	NR	2015	4-16	Primary and Permanent	mDDE	Teeth examined wet and undried except few subjects cleaning was carried out; chronologic enamel hypoplasia most prevalent, no statistically significant difference in distribution of defects between males and females.	11.2	NR
Masumo et al. (2013)	Tanzania	NR	1221	0.6-3	Primary	mDDE	Dentists undertook dental examination, teeth cleaned and dried; diffuse opacity most common defects, followed by hypoplasia and then demarcated opacity. Max central incisors most frequent teeth affected whereas lower central incisors least frequently affected. Normal birth associated with lower odds of having enamel hypoplasia.	33.3	NR
Multi-country									
Nunn et al. (1993)	Sri Lanka England	0.1 0.5 1.0	547	12	Permanent	mDDE	Natural light and undried buccal surfaces; demarcated and diffuse opacities predominated in the 1ppm F areas.	NR	NR
(Balmer et al., 2005)	UK Australia	<0.1 0.9-1.1	24	8-16	Permanent	mDDE	Higher prevalence of defects among children living in fluoridated than non fluoridated areas.	NR	27.3 51.6

Table 2-3: Studies describing prevalence and pattern of distribution of developmental defects of enamel among children.

*Notes: Prev. - Prevalence; NR- Not reported; mDDE – Modified developmental defects of enamel; DO – Diffuse opacities; H – Hypoplasia; FA – Fluoridated area; NFA – Non-fluoridated area; MIH – Molar-incisor hypomineralization; n – number; * - Mean number of teeth affected; Max. – Maxilla; Mand. - Mandible*

Table 2.3 shows that the mean number of permanent teeth affected by DDE ranged from 1.98 among Brazilian 7 – 13 year olds (Soviero et al., 2009) to 3.6 in an Australian study of children (Seow et al., 2011) although age was not reported in the latter study. The differences in the tooth prevalence of DDE in both primary and permanent teeth might be due to the differences between the age groups, geographical location and aetiological factors of DDE.

Some studies have reported diffuse opacities as the most prevalent type of enamel defects in primary teeth (Lunardelli and Peres, 2005, Masumo et al., 2013, Robles et al., 2013) and permanent teeth (Wong et al., 2006, Yusoff et al., 2008, Vargas-Ferreira et al., 2014) while others have reported hypoplasia as the commonest enamel defects in primary teeth (Li et al., 1995, Kanchanakamol et al., 1996, Lin et al., 2011) and permanent teeth (Aine et al., 2000, Seow et al., 2011). In contrast some studies have shown that demarcated opacities were the most prevalent enamel defects in primary teeth (Farsi, 2010, Corrêa-Faria et al., 2013b)and permanent teeth (Wogelius et al., 2008, Soviero et al., 2009, Robles et al., 2013). A recent study in Brazilian infants (Gravina et al., 2013) reported that opacities, whether demarcated or diffuse, occur more than hypoplasia in the primary teeth of children studied. The observed differences in the prevalence of the types of DDE in these primary teeth might be due to differences in the examination conditions and the causative factors. Diffuse opacities have been reported to be associated with the F concentration in drinking water and it is reported to be the feature distinguishing between the teeth of children living in low and high fluoridated areas (Cutress et al., 1985). Demarcated opacities and hypoplasia have been shown to be associated with trauma to developing tooth bud especially among preterm babies during intubation (Franco et al., 2007).

There is some variation between studies over the distribution of DDE according to tooth type. In the primary dentition, molars especially second molars (Li et al., 1995, Slayton et al., 2001, Lunardelli and Peres, 2005), upper central incisors (Cruvinel et al., 2012, Gravina et al., 2013), lower central incisors (Masumo et al., 2013) were the teeth most affected by developmental defects. In the permanent teeth, upper central incisors (Dummer et al., 1990, Wogelius et al., 2008), upper canines and premolars (Nunn et al., 1993) and first molars (Soviero et al., 2009) were mostly affected by DDE. These differences in the pattern of distribution of DDE in the primary dentition should be interpreted with caution since, depending on the age group studied a partially or fully erupted dentition may be examined. In addition, these differences may be related birth trauma to anterior tooth buds and to the

diagnosis between defects in the enamel of incisors and molars and early caries in infancy. Some studies have reported that DDE is symmetrically distributed in the primary teeth (Slayton et al., 2001, Lin et al., 2011) as well as in permanent teeth (Yusoff et al., 2008) of children since systemic causative factors affect the same group of primary or permanent teeth on both sides of the maxillary and mandibular arches at a particular time of development. On the contrary, a large study (Dummer et al., 1990) on tooth type and surface in developmental defects of enamel in 791 fifteen to sixteen year-old Wales children reported that maxillary lateral incisors were significantly more distributed to the right while maxillary first molars were significantly more distributed to the left side of the mouth, although the reasons for this were unclear.

Other cross-sectional observational studies have reported that DDE occur more in the maxilla than mandible (Dummer et al., 1990, Lunardelli and Peres, 2005, Kar et al., 2014), on buccal surfaces more than other surfaces (Dummer et al., 1986, Li et al., 1995), in children who attend public schools more than those who attend private schools and in females more than males (Memarpour et al., 2014). On the contrary, a study of 1344 five year-olds in China (Li et al., 1995) reported that DDE affected more males than females while studies in USA and Nigeria (Slayton et al., 2001, Orenuga and Odukoya, 2010) reported no gender difference. These differences seen between studies might be due to diversity in the methodological procedures including the sampling and racial differences. Developmental defects of enamel have also been shown to be more prevalent among preterm babies than full term babies (Gravina et al., 2013, Masumo et al., 2013) and low birth weight babies (LBWB) than normal birth weight babies (Corrêa-Faria et al., 2013a, Memarpour et al., 2014). The occurrence of DDE among preterm and LBWB may be due to increased risk of infections (Franco et al., 2007), poor feeding and lack of optimal nutrition (Jacobsen et al., 2014) as well as trauma from oral intubation and long term mechanical ventilation (Seow et al., 1984). The presence of childhood illnesses (Gravina et al., 2013, Memarpour et al., 2014) and malnutrition (Rugg-Gunn et al., 1998) have also been associated with the occurrence of DDE due to inability of the body to absorb calcium that is needed for enamel formation and development. Similarly, a lack of breastfeeding has been shown to be related to the development of DDE (Vignarajah and Williams, 1992, Lunardelli and Peres, 2006) and thought to be due to poor immunity that might result in gastro-intestinal malabsorption of essential vitamins and minerals required for enamel formation.

The prevalence and severity of developmental defects of enamel in the primary and permanent teeth varies between various groups of children exposed to different water F concentration in the same and/or different countries, although most publications did not state tooth prevalence of the defects which is important in determining the extent of the condition in the mouth and the severity of the disease condition in a population. There is scarcity of literature on the pattern and distribution of defects in primary teeth as compared to permanent teeth with only a few reports regarding the effect of chemicals and drugs on the primary dentition. There is also a dearth of literature on the occurrence of DDE in Africa, especially sub-Saharan Africa.

2.7 Epidemiology of Dental fluorosis (Table 2.4)

Globally, dental fluorosis is endemic in at least 25 countries and a conservative estimate of the total number of people affected would number tens of millions (Arvind et al., 2012). Dental fluorosis occurrence has become more widespread and its prevalence has increased even in areas with F-deficient water supplies (Leverett, 1986); the increased exposure to fluorides from other sources has been suggested as the possible explanation (Akpata et al., 1997). Fluorides from beverages, toothpastes, infant formula, F supplements, food condiments and sea foods may be responsible for the occurrence and high prevalence of dental fluorosis in these water F-deficient with these sources of F contributing to increased background F exposure (Pang et al., 1992, Mabelya et al., 1992, Fejerskov et al., 1994). The prevalence of dental fluorosis varies widely among populations and is reported to be due to several factors including the range of concentrations of F found in various dietary sources, quantities of water consumed, malnutrition, genetics, gastric acidity and kidney function.

Table 2.4 shows the prevalence and pattern of distribution of dental fluorosis among children in various countries. The majority of the studies were undertaken in Africa especially in East African countries, due to presence of high water and soil F concentrations from granites and gneissic rocks and volcanic activities in these countries. TFI was the main index used to measure the presence of dental fluorosis in the majority of the studies because it gives more complete information on the distribution of dental fluorosis within the dentition since each tooth is scored. In addition, TFI also gives better understanding of the relationship between dental fluorosis and an individual‘s exposure to F during tooth formation.

The majority of the studies used a direct clinical examination rather than a photographic method to detect and assess dental fluorosis while 3 studies (Tavener et al., 2004, Cochran et al., 2004a, Tavener et al., 2006) used the latter. Direct clinical dental examinations are relatively quick, of low cost and allow both visual and tactile examinations to be employed. Photographic methods were utilised in a multicentre study (Cochran et al., 2004a) to determine the prevalence and severity of dental fluorosis among 8 year-olds in 7 European countries where it was important to ensure a reproducible, reliable and efficient means of collecting similar data across a number of countries. In this study images of the teeth were scored remotely using TFI. Table 2-4 describes the prevalence and pattern of dental fluorosis reported in studies identified between 1987 and 2014. Most of these cross-sectional observational studies have involved the permanent dentition; mainly because dental fluorosis in this dentition is seen to be more of a public health problem. In addition, primary teeth exfoliate making fluorosis in primary teeth often seen to be of little importance (Browne et al., 2005). Fluorosis prevalence is lower in primary teeth because they start to develop *in-utero* and the placenta protects them from excessive F exposure. In addition, their calcification is usually well advanced when they start to be exposed to excessive F from drinking water or other sources (Table 2-1). Dental fluorosis is more common in permanent teeth because their calcification is often just commencing when they start to be exposed to excessive F intake resulting in fluorosis. Some studies (Milsom et al., 1996, Levy et al., 2006) have suggested that fluorosis in primary teeth can have an association with fluorosis development in the permanent teeth but there is lack of sufficient evidence for the risk factors. However, to avoid occurrence in permanent teeth, it is important to investigate possible sources of F ingestion in infants and younger children exhibiting dental fluorosis in the primary dentition (de Carvalho et al., 2013).

The reported prevalence of dental fluorosis in primary and permanent teeth varies widely from one age group and country to another because of the use of various terminologies and the different diagnostic criteria employed to describe dental fluorosis. The comparison of prevalence of dental fluorosis in primary dentition among studies is very difficult due to the few studies published and the diversity of indices used to evaluate it (de Carvalho et al., 2013). No study has reported tooth prevalence of dental fluorosis in primary teeth and globally the mouth prevalence of dental fluorosis in primary teeth has been shown to range from 0% reported for Swedish children who lived in a <0.2 ppm F water area (Forsman, 1977) to 96.5% in 7 – 8 year-old Chinese children who lived in a 7.6 ppmF water area (Ruan et al., 2005a).

Authors (date)	Country	F level (ppm)	Age (Years)	n	Dentition	Index	Exam conditions and Notes	Results	
								Mouth Prev. (%)	Tooth Prev. (%)
Europe									
(Forsman, 1977)	Sweden	< 0.2	NR	NR	Primary	Dean	Very mild or mild fluorosis occurred at higher F concentrations	0	NR
Hawley et al. (1996)	England	NR	14	435	Permanent	TFI	The subjects who had no fluorosis had a mean DMFT of 3.0 which was significantly higher than the mean of 2.2 among those with any fluorosis	11	NR
Rock and Sabieha (1997)	England	1.0	8-9	325	Permanent	TFI	Highly significant associations were found between estimated fluoride ingestion from toothpaste and fluorosis. The mean DMF score of the fluorosis group was half that of the fluorosis-free children	34.4	NR
Bårdsen et al. (1999)	Norway	0.1 ≥0.5	5-18	105 113	Primary & Permanent	TFI	Teeth cleaned and dried; Premolars were most frequently affected; only fluoride concentration in the drinking water was associated with a statistically increased risk of dental fluorosis	14.3 78.8	NR
Tabari et al. (2000)	England	1.0 <0.1	8-9	409 403	Primary & Permanent	TFI	Teeth wiped with cotton roll under daylight; the risk factors were--fluoridated area, affluence, and use of adult toothpaste.	54 23	NR
Tavener et al. (2004)	England	<0.1+1450TP <0.1+440TP <0.1	8-9	703	Permanent	TFI	Wet and dry teeth images; For both the wet and dry photographs the prevalence of any enamel defects was similar for the three groups	17^{x} – 26^{y} 15^{x}-24^{y} 12^{x}-25^{y}	NR
(Harding et al., 2005)	Ireland	Fluoridated Non-fluoridated	5	208 86	Primary	Modified TSIF	Factors that were associated with primary tooth fluorosis were: fluoridation status and the age at which tooth brushing with toothpaste commenced. No association with infant feeding practices was identified.	32 1	
Tavener et al. (2006)	England	<0.1+1450TP <0.1+440TP	8-10	1268	Permanent	TFI	Dries teeth images; There was a strong association between the deprivation status of wards and fluorosis.	25.2^{a}-34.5^{b} 19.5^{a}-23.7^{b}	NR

Authors (date)	Country	F level (ppm)	Age (Years)	n	Dentition	Index	Exam conditions and Notes	Results	
								Mouth Prev. (%)	Tooth Prev. (%)
Narbutaite et al. (2007)	Lithuania	High F Low F	12	600	Permanent	TFI	The mean number of teeth with fluorosis was 4.5 for the High F group and 0.2 for the Low F group. In the High F group, 72% had a decayed, missing or filled teeth (DMFT) score of > 0, compared with 87% in the Low F group	66 4	NR
North America									
Osuji et al. (1988b)	Canada	1.0	8-10	633	Primary & Permanent	TFI	Those who brushed their teeth before the age of 25 months had 11 times the odds of fluorosis compared with those beginning tooth brushing later; prolonged use of infant formula (greater than or equal to 13 months) was associated with 3.5 times the risk of fluorosis, compared with no, or shorter duration of, formula use	12.9	NR
Szpunar and Burt (1988)	USA	0.8 1.0 1.2 0.0	6-12	425 131	Primary Permanent	TSIF	The odds of experiencing fluorosis increased at every F level above the baseline, with the use of topical F rinses, and with age.	31 49 51.2 12.2	NR
Kumar et al. (1989)	USA	1.0 <0.3	7-14	539 510	Primary & Permanent	Dean's	10 years of fluoridation both fluoridated and non-fluoridated areas revealed no changes in dental fluorosis prevalence in the former while changes in dental fluorosis prevalence was apparent in the latter.	7.7 7.4	NR
Ismail et al. (1990)	Canada	<0.1	11-17	499	Permanent	TSIF	The risk factors for dental fluorosis were the use of fluoridated water and fluoride tablets.	31	NR
Heller et al. (1997)	USA	> 1.2 0.7-1.2 0.3 – 0.7 <0.3	7-14	6728 6239	Primary & Permanent	TSIF	The use of fluoride supplements was associated with both lower caries and increased fluorosis.	41.4 29.9 21.7 13.5	NR

Authors (date)	Country	F level (ppm)	Age (Years)	n	Dentition	Index	Exam conditions and Notes	Results	
								Mouth Prev. (%)	Tooth Prev. (%)
Jackson et al. (1999)	USA	1.0 0.2	7-14	122 124	Primary Permanent	TSIF	The prevalence of fluorosis increased by approximately 14%, 20%, and 6% in the negligibly, optimally, and 4X optimally fluoridated communities, respectively.	58 42	NR
(Warren et al., 2001)	USA	NR	4.5 – 5	637	Primary	Modified TSIF	Nearly all fluorosis was mild, with the primary second molar teeth most commonly affected. Fluorosis was significantly associated with higher water fluoride concentration, but not with the use of dentifrice or fluoride supplements.	11.6	
South America									
Molina-Frechero et al. (2012)	Mexico	0.3	11	111	Permanent	Modified Dean's & CFI	Children who had no fluorosis showed more caries. Dental fluorosis was associated with the initial age of brushing (before age four), frequency of brushing (three times a day), brushing before sleeping (yes) and applications of fluoride (yes).	52.7	NR
García-Pérez et al. (2013)	Mexico	0.7 1.5	8-12	457	Permanent	TFI	Cleaned and teeth dried; A logistic regression model for caries showed that higher fluorosis categories were associated with higher caries experience	39.4 60.5	NR
(de Carvalho et al., 2013)	Brazil	0.6-0.8	4-6	315	Primary	Dean's	Natural light and teeth dried; Dental fluorosis in primary teeth was associated with lactose intolerance but there was no significant association with the use of manufactured soy-based products.	11	
Australasia									
Riordan and Banks (1991)	Australia	0.8 <0.2	12	338 321	Permanent	TFI	Teeth dried, Increasing exposure was associated with higher fluorosis prevalence and higher TF scores,	40.3 30	0.40 0.33
Asia									
Ruan et al. (2005b)	China	1.2	10-11		Permanent	TFI	Storage of water in clay pots seemed to increase the severity of fluorosis slightly, and to decrease the caries prevalence.	50	

Authors (date)	Country	F level (ppm)	Age (Years)	n	Dentition	Index	Exam conditions and Notes	Results	
								Mouth Prev. (%)	Tooth Prev. (%)
Ruan et al. (2005a)	China	0.4-7.6	7-8	472	Primary	TFI	The second primary molars were most severely affected by dental fluorosis. Dental fluorosis was symmetrically distributed in both jaws.	6.2-96.6	
Shanthi et al. (2014)	India	NR	9-12	1500	Permanent	WHO guidelines	Number of children having dental fluorosis was highest in children who consume water from bore wells. Caries prevalence and mean DMFT/dmft scores were least in children with optimal F areas and highest in children with below optimal F areas.	74	NR
Middle East									
Akpata et al. (1997)	Saudi Arabia	0.5-2.8	12-13	2355	Permanent	TFI	Teeth dried & pocket torch; a strong association between fluoride level in well drinking water and severity of dental fluorosis	>90	NR
Ramezani et al. (2004)	Iran	2.4	13±3	506	Permanent	NR	Severe fluorosis and mean DMFT was higher in girls than boys	71.1%	NR
Meyer-Lueckel et al. (2011)	Iran	0.3 3.2	12-16	373	Permanent	TFI	Water fluoride concentration was inversely associated with caries-status	1 87	NR
Poureslami et al. (2013)	Iran	Fluoridated	5-6	272	Primary	TSIF	The average fluoride content of the enamel and coronal dentin was 108.7 µg/mL.	76.5	NR
Africa									
Haimanot et al. (1987)	Ethiopia	1.1-3.6	10-14	NR	Permanent	TFI	Males were affected more than females; skeletal fluorosis observed in some areas	80	NR
Ng'ang'a and Valderhaug (1993)	Kenya	Non-fluoridated	6-8	513	Primary Permanent	TFI	Natural light; There was no significant sex difference in either the prevalence or the severity of fluorosis.	18 76	NR
Ibrahim et al. (1995)	Sudan	0.25 2.56	7-16		Permanent	Dean's & CFI	In both areas great inter-individual variations in dental fluorosis were recorded.	91 100	NR
Lewis and Chikte (1995)	South Africa	0.6-1.6 8.9	6-18	262	Permanent	TSIF	The population prevalences of fluorosis were similar in the 2 areas but significant differences existed in severity.	>90	60 78

Authors (date)	Country	F level (ppm)	Age (Years)	n	Dentition	Index	Exam conditions and Notes	Results	
								Mouth Prev. (%)	Tooth Prev. (%)
Mabelya et al. (1997)	Tanzania	0.2-0.8	12-17	1566	Permanent	TFI	The urinary fluoride excretion corresponded with the level of fluorosis and the fluoride content in the magadi samples.	7-46[p] 53-100[q]	NR
El-Nadeef and Honkala (1998)	Nigeria	0.0-0.04	12-15	203	Permanent	TFI	The majority had very mild fluorosis	51	NR
Awadia et al. (1999)	Ethiopia	3.6	6-18	24 141	Permanent	TFI	Vegetarianism and a series of other factors related to childhood nutrition were significantly associated with the tooth prevalence of dental fluorosis.	67[o] 95[m]	NR
Awadia et al. (2000a)	Tanzania	0.2 3.6	8-16	96 80	Permanent	TFI	The severity, however, was significantly higher in Arusha.	99 96	NR
Wongdem et al. (2001)	Nigeria	Fluoridated Non-fluoridated	7-19	475	Permanent	TFI	There was a markedly significant association between fluorosis and source of drinking water	26.1	NR
Grobler et al. (2001)	South Africa	0.19 0.48 3.0	10-15	282	Permanent	Dean's	A strong positive correlation was found between the caries experience and the fluorosis scores of children in the high F area but no correlation could be found in the low F areas. Significantly more children had decayed teeth in the high F area than in the other two areas.	47[z] 50[z] 95[z]	NR
Wondwossen et al. (2003)	Ethiopia	NR	12-15	306	Permanent	TFI	The child/mother pairs found teeth with TF scores 2 and 3 aesthetically acceptable, while teeth with TF scores 5 and 7 were considered unacceptable. Mothers were more critical of severe fluorosis than were their children.	72	NR
Wondwossen et al. (2006)	Ethiopia	0.3-2.2 10-14	12-15	233	Permanent	TFI	The odds for having severe fluorosis varied according to the fluoride concentration of the drinking water, age, consumption of tea, length of breastfeeding and method of storing water.	24.1* 75.9*	NR

Authors (date)	Country	F level (ppm)	Age (Years)	n	Dentition	Index	Exam conditions and Notes	Results	
								Mouth Prev. (%)	Tooth Prev. (%)
Zerihun et al. (2006)	Ethiopia	Fluoridated	11-14	472	Permanent	TFI	Children born in the fluoridated area compared to those born elsewhere; males compared to female; and those who obtained their regular drinking water from pipe distribution compared to those who obtained water from other sources were more likely to have dental fluorosis.	84	NR
Akosu et al. (2009)	Nigeria		12-15	1100	Permanent	TFI	Significantly higher prevalence of dental fluorosis (22.2%) in the high altitude areas compared to the low altitude ones (3.5%).	12.9	NR
Vuhahula et al. (2009)	Tanzania	1.5-24.9	12-18	2912	Permanent	TFI	A total of 83.3% of children had at least one tooth with TFI score≥4.	96.3	NR
Shorter et al. (2010)	Tanzania	>1.5	School children	275	Primary & Permanent	TSFI	Deformities relating to SF are common, but the reasons for individual susceptibility remain unclear and may include a low calcium diet, ingestion of magadi (local salt) with high fluoride, or genetic factors.	>90	NR
Rango et al. (2012)	Ethiopia	7.8-18	7-40	200	Primary & Permanent	TFI	Sixty percent of the teeth exhibited loss of the outermost enamel. No correlation between fluoride content and DF. Milk intake contributed to reducing the severity of dental fluorosis.	100	NR
Firempong et al. (2013)	Ghana	<1.0 >1.5	7-18	200	Primary & Permanent	Dean's	Statistically, there was no significant relationship between the presence of dental fluorosis and the other characteristics, except the age group and fluoride ion concentration of the area.	10 63	NR
Multi-country									

Authors (date)	Country	F level (ppm)	Age (Years)	n	Dentition	Index	Exam conditions and Notes	Results	
								Mouth Prev. (%)	Tooth Prev. (%)
Cochran et al. (2004a)	Ireland England Finland Greece Iceland Netherlands Portugal	1.0 <0.1 <0.01 <0.01 0.05 0.13 0.08	8	325 314 315 283 296 303 210	Permanent	TFI	Dried teeth images; Fluoridated water and the prolonged use of fluoride tablets were found to be significant contributory factors to fluorosis.	26.2 20.7 26 18.7 23 26.4 24.3	NR

Table 2-4: Prevalence and pattern of distribution of dental fluorosis among children.

*Notes: n – Number; Prev. – Prevalence; NR – Not reported; TFI – Thystrup & Fejerskov index; TSFI - Tooth surface fluorosis index; CFI – Community fluorosis index; NR – Not reported; TP – Toothpaste ; [x] – wet; [y] – dry ; [a] – deprived; [b] – less deprived; * - severe dental fluorosis TF ≥5; [o] – vegetarian; [m] – non-vegetarian; [p] – no magadi; q – magadi consumption; p – prevalence; DMFT – Decayed missing filled teeth; DMFS – Decayed missing filled surface; DF – Dental fluorosis; DC – Dental caries; [z] – Dean's score 2, 3 and 5.*

Two studies reported tooth prevalence of dental fluorosis in permanent teeth; one reported it as proportion – 60% and 78% of teeth affected among 6 to 18 year-old South African children living in 0.6 - 1.6 ppmF and 8.9 ppmF areas respectively (Lewis and Chikte, 1995) while an Australian study reported it as mean of 0.33 and 0.40 teeth affected among 12 year-olds living in 0.8 ppmF and <0.2 ppmF areas respectively (Riordan and Banks, 1991). Worldwide, mouth prevalence of dental fluorosis in permanent teeth range has been reported as 1% for 12 to 16 year-old Iranian children living in 0.3 ppm water areas (Meyer-Lueckel et al., 2011) to 100% reported for 7 to 16 year-old Sudanese children living in 2.56 ppm water F areas (Ibrahim et al., 1995) and for 7 to 40 year-old Ethiopians living in 7.8 to 18 ppm F water areas (Rango et al., 2012). It is not surprising to observe that the prevalence of dental fluorosis was 100% in the studies undertaken in Sudan and Ethiopia since the water F concentrations were high and exceeded 2ppm F in both study locations. Overall, the differences in the prevalence of dental fluorosis in both primary and permanent dentition might be due to differences in age group studied, methodological procedures, dietary and tooth cleaning practices.

A positive correlation between F concentration in water and the prevalence of dental fluorosis especially in permanent teeth was demonstrated in a number of studies (Wondwossen et al., 2003, Meyer-Lueckel et al., 2011, García-Pérez et al., 2013) and further demonstrated by the association found between powdered infant formula reconstituted with water and dental fluorosis indicating the effect of water F used for preparing infant formula and the F content of infant formula itself (Bårdsen et al., 1999, Levy et al., 2010). In areas of the world with natural F in water sourced from wells, the prevalence of dental fluorosis also depends on the depth of the wells, with water from deeper wells providing a higher F concentration (Akpata et al., 1997). However, contrary to this clear relationship, a study (El-Nadeef and Honkala, 1998) in central Nigeria reported that about 50% of children aged 12 to 15 years had dental fluorosis in an environment where F concentrations in drinking water were low and that factors other than F concentrations of water such as toothpaste ingestion, diet and genetics might have contributed to the severity of dental fluorosis.

When teeth surfaces are dried before employing a direct clinical or photographic examination, assessment of these surfaces for the presence of enamel defect is enhanced. This was confirmed in a study (Tavener et al., 2004) on prevalence and severity of fluorosis in children who received free F toothpaste in which the prevalence of dental fluorosis was higher among children whose teeth were dried before their images were photographically

taken than those whose teeth were not dried. Drying of teeth surfaces before taking their images ensure that the surfaces were not blurred by saliva or debris. In addition, the prevalence of dental fluorosis among 8 to 16 year-old Ethiopian children living in 3.6 ppm water F areas was found to be higher among non-vegetarian children than vegetarian children (Awadia et al., 1999) because vegetarian diets increase gastric emptying resulting in decreased F absorption in the stomach.

Based on the distribution of dental fluorosis in the primary teeth, second primary molars were reported as the teeth mostly affected (Warren et al., 2001, Ruan et al., 2005a) because they are the last teeth to calcify thereby exposing them post-natally to the effect of F from water or toothpaste when the child starts drinking water or tooth brushing. Conversely, a study (Ng'ang'a and Valderhaug, 1993) on prevalence and severity of dental fluorosis among 6 to 8 year-old Kenyan children reported no difference between in both anterior and posterior primary and permanent teeth. Based on gender distribution, there was no significant difference in the occurrence of dental fluorosis in the primary (Ruan et al., 2005a) and permanent (Wongdem et al., 2001, Azevedo et al., 2014) dentitions of males and females. However, it occurred more in primary (Mann et al., 1990, Warren et al., 1999, Narwaria and Saksena, 2013) and permanent (Zerihun et al., 2006, Wondwossen et al., 2006) teeth in males than females in some studies while it occurred more in the permanent teeth of the latter than former (Ramezani et al., 2004). The reason for these observed gender differences might be due to differences in the index used in assessing dental fluorosis as well as the geographical locations of the studies.

The enamel changes within the primary and permanent dentitions exhibited the expected bilateral symmetry characteristics of dental fluorosis in some studies (Ng'ang'a and Valderhaug, 1993, Ruan et al., 2005a). This is because tooth forming cells and organs develop bilaterally at same time thus are affected symmetrically by systemic aetiological factors. Generally, in young children excessive F ingestion from repeated swallowing of toothpaste is associated with dental fluorosis in both primary and permanent teeth (Rock and Sabieha, 1997, Tabari et al., 2000). This is particularly so in children younger than 2 years who may intentionally (and inadvertently) ingest toothpaste due to its minty and sweet flavour. Studies have shown that dental fluorosis in the primary (Harding et al., 2005) and permanent (Molina-Frechero et al., 2012) teeth was significantly associated with age at which tooth brushing with toothpaste commenced; children who commenced tooth brushing earlier having more fluorosis than their counterparts. In addition, the use of adult toothpaste (Tabari et al., 2000), frequency of tooth brushing and brushing before sleeping

(Molina-Frechero et al., 2012) have been demonstrated to be associated with dental fluorosis in both primary and permanent teeth (Tabari et al., 2000). Ingesting adult toothpaste could increase the risk of fluorosis in children because it usually contains higher concentration of F than a child's toothpaste formulation. Although, increase in the number of times of tooth brushing and brushing before bed could enhance the oral hygiene of children it would result in increased swallowing of toothpaste especially among young children thereby resulting in fluorosis.

Prolonged use of F supplements has been reported to be associated with fluorosis in permanent teeth (Riordan and Banks, 1991, Cochran et al., 2004a) because of their relatively high F concentrations when ingested. The prevalence and severity of dental fluorosis among 8 to 10 year-olds was associated with the deprivation status of wards in England (Tavener et al., 2006), it was higher among children who lived less deprived areas than those who lived in deprived areas. Similarly, the prevalence of fluorosis has been reported as higher among children who attend private school (de Carvalho et al., 2013) and in those from a higher socio-economic group than their counterparts (Tabari et al., 2000). Attending private school and living in less deprived areas is probably a sign of affluence which might translate into greater exposure to the use of F in its various forms and consequently dental fluorosis. Dental fluorosis in the permanent teeth of children has also been associated with tea consumption, length of breastfeeding and method of storing water (Wondwossen et al., 2006). In this study, children who did not consume tea, who were breastfed for more than 18 months and those who were brought up in households using clay pots for storage of the daily water supply were less prone to develop severe dental fluorosis. Some tea plants are known to accumulate F which is easily released into water thereby complementing the effect of F in water on developing tooth bud and the risk of developing fluorosis could increase if tea is drank daily. Prolonged breastfeeding, especially during the first one or 2 years of life which coincide with periods of active enamel formation, protects against dental fluorosis. Clay pots especially red lateritic clay pots have been shown to remove F from high F water (Bjorvatn et al., 2003). Living at high altitude (Akosu et al., 2009) and the consumption of a food tenderiser or trona in some parts of Tanzania (Magadi) (Mabelya et al., 1997, Vuhahula et al., 2009) have also been shown to be related to the presence of dental fluorosis among children. In terms of the effects of altitude, alterations in acid-base balance caused by hypobaric hypoxia during residence at high altitude were cited as the cause of decreased urinary pH, reduction in renal F excretion and therefore increased F body burden (Whitford, 1997). A significant higher prevalence of

fluorosis was observed in Tanzanian communities at a high altitude of 1,463 m in contrast with a low altitude area of 100 m but with similar food habits and low F in drinking water (Yoder et al., 1998). Studies conducted in other countries have confirmed this finding (Rwenyonyi et al., 1999, Martínez-Mier et al., 2004, Pontigo-Loyola et al., 2008, Akosu and Zoakah, 2008) suggesting that physiological changes associated with residence at high altitude are able to exacerbate the effects of F in mineralized tissues. It has also been reported that residence at high altitude can have profound disruptive effects on amelogenesis that can be confused with dental fluorosis (Angmar-Mansson and Whitford, 1990). Magadi, a meat and vegetable tenderiser used to speed up the cooking time of foods to help conserve fuel, is used in numerous places in Tanzania to contain high levels of F. Exposure to it in some Tanzanian communities has been reported as the cause of severe dental fluorosis in communities with low water F (Mabelya et al., 1992).

In summary, globally there is a wide variation in the prevalence and severity of dental fluorosis in both primary and permanent teeth among children. The Thystrup and Fejerskov fluorosis index was the tool mostly used for assessing the presence of dental fluorosis. The majority of the studies reported dental fluorosis involving permanent dentition than primary dentition and direct clinical examination was the primary method used for measuring dental fluorosis. A number of environmental factors were identified as possible aetiological factors in the occurrence of dental fluorosis.

2.8 Relationship between developmental defects of enamel, dental fluorosis and dental caries

Enamel defects have attracted increasing attention with studies linking enamel defects with dental caries in the primary and permanent dentitions (Hong et al., 2009, Carvalho et al., 2011, Targino et al., 2011). Some researchers have reported that DDE may be a risk factor for Early Childhood Caries (ECC) since the retentive areas created by the structural defects can lead to build up of bacterial plaque, thereby facilitating caries progression (Oliveira et al., 2006, Targino et al., 2011). In addition, more mutans streptococci, which are associated with the aetiology of dental caries, are found among children with DDE (Shanmugam et al., 2013). It has been demonstrated that in primary teeth, the area of enamel affected by defects (hypomineralisation) has a lower mineral content compared to sound enamel and is similar to the mineral content in white spot lesions (Elfrink et al., 2013b).

This lower mineral content can allow additional plaque accumulation and Streptococcus mutans (Milgrom et al., 2000) and Lactobacilli colonisation thereby enhancing caries onset and progression compared with non-defective enamel (Caufield et al., 2012). Studies on the association between developmental defects of enamel and early childhood caries, showed that DDE was associated with the occurrence of ECC in the primary dentition, as the majority of the children with DDE had at least one tooth with decay while those without DDE had no teeth with decay (Oliveira et al., 2006, Corrêa-Faria et al., 2014).

Despite the observed association between DDE and ECC and the identification of susceptible children, few studies (Milgrom et al. 2000; Montero et al. 2003; Ribeiro et al. 2005; Farsi et al. 2010) have addressed the influence of DDE on the occurrence of ECC among younger children especially of preschool age.

Despite the consensus on the association between DDE and dental caries, comparisons among studies need to be conducted with caution due to methodological differences (Corrêa-Faria et al., 2014). Assessment tools used for the diagnosis of DDE differ between studies; while some authors have used the DDE index (Kanchanakamol et al., 1996, Ribeiro et al., 2005) others have used the mDDE (Montero et al., 2003, Farsi, 2010). Also, some studies (Milgrom et al., 2000, Vargas-Ferreira et al., 2014) only investigated the association between ECC and quantitative defects on enamel (hypoplasia) while some (Farsi, 2010, Carvalho et al., 2011, Corrêa-Faria et al., 2014) studied the association among the three types of DDE (hypoplasia, demarcated opacities and diffuse opacities). With an increased risk of caries due to hypoplasia, Caulfield and co-workers (Caufield et al., 2012) proposed a new classification of severe early childhood caries which included enamel hypoplasia since primary teeth with enamel hypoplasia are more vulnerable to early and greater colonisation by cariogenic bacteria. A cross-sectional study (Vargas-Ferreira et al., 2014) undertaken to determine the association between DDE and dental caries among 8 to 12 year old Brazilian school children showed that caries was more common among children who had enamel hypoplasia in their posterior teeth than among those with none while there was no association in the anterior teeth.

The widespread use of F in its various forms has resulted in decrease in the prevalence and severity of dental caries, however excessive intake of F during tooth development can cause dental fluorosis. To minimise the risk of dental fluorosis and maximise the benefit of F, optimal amounts of F should be administered to children during tooth development. Cross-sectional studies on dental caries and dental fluorosis in some part of the world have

reported an inverse relationship between their prevalence and severity (Mascarenhas and Mashabi, 2008, Kotecha et al., 2012, Punitha et al., 2014); dental caries prevalence generally being less in children with dental fluorosis. In a study on dental fluorosis and dental caries among 12-year old children from high and low F areas in Lithuania, Narbutaite and colleagues reported that high F concentration in drinking water may increase the prevalence and severity of dental fluorosis and decrease the occurrence of caries (Narbutaite et al., 2007). On the contrary, some studies (Grobler et al., 2001, Awadia et al., 2002, Wondwossen et al., 2004) on the relationship between dental caries and dental fluorosis in different drinking water F levels showed that dental caries increased with increasing severity of dental fluorosis in both areas. These studies further showed that dental caries experience in the permanent dentition increases significantly with increasing F content in drinking water. There is a need to further study the relationship between dental fluorosis and dental caries since both conditions impact negatively on the quality of life of children. A study that compared impacts on oral health related quality of life associated with dental caries and fluorosis among 174 rural Ugandan children showed that the two disease conditions impacted negatively on the children who had them, however, the greater burden on oral health related quality of life was associated with dental caries.

In summary, DDE such as hypoplasia is a risk factor for dental caries since the disease condition encourages the pathogenesis of dental caries while there is controversy over the relationship between dental fluorosis and dental caries. Therefore, further studies should be undertaken to investigate the relationship between these two diseases conditions with a view to ameliorating negative impacts on the quality of life of children.

2.9 Systemic F intake

Fluorides are found in various sources since they occur naturally in soil, water, plants, animals and humans in trace quantities (Mandinic et al., 2010). Children may be exposed to high doses of F due to various sources of ingestion which could increase their risk of developing dental fluorosis (de Carvalho et al., 2013). The main sources of both systemic and local exposure to F and risk factors are: fluoridated water, infant formulas, supplements, fluoridated dentifrices and infant foods and beverages (Mascarenhas, 2000, Buzalaf et al., 2004). These sources of F exposure are classified into dietary and non-dietary sources.

2.9.1 Dietary sources of systemic F exposure

It is important to know the F content of the drinks and foods consumed by children at the age when they are at increased risk of developing dental fluorosis (de Carvalho et al., 2013) during critical periods of tooth development, and developing skeletal fluorosis during critical periods of bone growth. In view of these critical "windows", infant feeding and, in particular, dilution of powdered milks, juices and infant formulas with fluoridated water can significantly contribute to high levels of F intake and should be considered an important source of F (Silva and Reynolds, 1996, Buzalaf et al., 2004).

2.9.1.1 Natural water fluoridation

Globally it is estimated that about 80% of diseases are attributed to poor quality drinking water and excessive F in drinking water is responsible for 65% of endemic fluorosis (WHO, 1984). Water is one of the major sources of F intake in humans and a prerequisite of sustainable development is to ensure uncontaminated drinking water sources, especially groundwater, worldwide (Narwaria and Saksena, 2013). The primary pathway by which people ingest excessive F is by constant consumption of high F water, when safe drinking water is not available (Mascarenhas, 2000) and the problem of excess F in water prevails in many countries of the world (Narwaria and Saksena, 2013). It has been estimated that about 200 million people, in 25 countries globally, are now under the threat of fluorosis since they are continually exposed to elevated F concentrations through drinking water (Ayoob and Gupta, 2006). This is thought to be due to growing water scarcity, high rates of population growth and adverse climatic changes seen in the recent past. The natural concentration of F in water depends on several contributing factors such as total dissolved solids and the porosity and acidity of the soil and rocks, as well as the temperature and depth of wells used to source water (Msonda et al., 2007, Viswanathan et al., 2009). Natural waters normally associated with high F are found in areas where F minerals are common and in calcium-deficient underground aquifers, geothermal streams, and in certain sedimentary basins (Teutli-Sequeira et al., 2011).

Large amounts of data are available regarding the F concentration of water supplies from various countries due to the ongoing interest in the benefits and disadvantages of F to human health. Fluoride is found in natural waters at low or high concentrations in areas with granitic and gneissic rocks and volcanic activity such as East African Rift system from Jordan valley down through Sudan, Ethiopia, Uganda, Kenya and the United Republic of Tanzania (Fawell et al., 2006, Shorter et al., 2010). Many of the lakes of the Rift Valley

system especially the soda lakes have extremely high F concentrations; 1,64mg/l and 2800 mg/l respectively in the Kenyan Lakes Elmentaita and Nakuru (Nair et al., 1984) and up to 690 mg/l in the Tanzanian Momella soda lakes (Fawell et al., 2006). High F in groundwater has been reported to be associated with igneous and metamorphic rocks from Iraq, Iran, Syria, Turkey, Algeria and Morocco India, Pakistan, West Africa, Thailand, China, Sri Lanka and Southern Africa (Fawell et al., 2006). Geographical mapping of F concentrations in drinking water sources from 109 randomly selected Local Government Areas (LGAs) in the 6 Nigerian Geopolitical zones showed that the F concentration was 0.3 ppm in 62% of the LGAs but in some sources it exceeded 1.5ppm and was as high as 6.7 ppm in one well (Akpata et al., 2009). The majority (91%) of drinking waters analysed in this Nigerian study were from natural groundwater. Further epidemiological research is needed to provide information about F concentrations from various ground water sources especially in urban and rural communities in Nigeria.

The United States Public Health Service in 1962 recommended a range from 0.7 to 1.2 mg/L as the optimum F concentration in drinking water depending on climate. When this guideline was followed in some countries especially tropical and subtropical countries of Africa and Asia such as Kenya, Senegal, Sri Lanka and Hong Kong, where water consumption is high, the prevalence and severity of fluorosis was found to be high (WHO, 1994b). In some of these countries new upper limits were recommended; for example 0.6 mg/L for Senegal and 0.8 mg/L for Sri Lanka and Hong Kong (WHO, 1994b, Ismail, 1995). General inadequacy of F exposure data in certain regions of the world and the tendency of certain nations to adopt and rely on 'blanket' WHO guidelines without regard to specific local F realities have not helped the situation (Wambu et al., 2014). The "optimum" or recommended concentration of F in drinking-water, associated with the maximum level of dental caries protection and minimum level of dental fluorosis, is considered to be approximately 1 ppm (Zemek et al., 2006, WHO, 2006a, Tamer et al., 2007), with 1.5 mg/L suggested as an acceptable upper limit which can vary depending on other factors such as climate and presence of other F sources..

Depending on the ambient temperature in different climates, Galagan and Vermillion (Galagan and Vermillion, 1957) suggested a formula for determining optimum F concentrations for community water supplies depending on climate and based on water intake which varies as the seasonal temperature changes.. Based on this formula, 0.6-0.7 ppmF has been recommended as appropriate for tropical countries like Nigeria and Saudi Arabia with mean maximum ambient temperature higher than 27^{o}C (Akpata et al., 2009,

Khan and Chohan, 2010). Geographical mapping of drinking water from all sources in Nigeria found that 21% of water sources naturally contain F to the recommended range of 0.3-0.6 ppm and about 62% have F below this range and the remaining 17% were above this range (Akpata et al., 2009). However, severe dental fluorosis has been observed in communities exposed to apparently appropriate F concentrations in drinking water in the tropics (Brouwer et al., 1988, Akpata et al., 1997) and this might be due to high water consumption or F exposure from other sources. Regular monitoring of F concentrations in community water to control community exposure to F is therefore highly recommended (Wambu et al., 2014) since the optimum water F concentration may not be the same for all geographical regions with the same temperature and may need to be adjusted for the water intake and dietary habits of different communities. It is important to determine F concentrations in drinking water sources in the community so as to prevent excessive F intake as well as guide decision making concerning F use.

The significant positive relationship between F intake from drinking water and the prevalence of dental fluorosis has been confirmed by several studies (WHO, 2006a, Mandinic et al., 2009, Viswanathan et al., 2009). Globally in fluorotic areas where endemic fluorosis has been well documented, F concentrations in drinking water range from 3 to more than 20mg/L and these high water F concentrations have been found in China, India and Africa (Cao et al., 2000b, Fantaye et al., 2004, WHO, 2006a, Tekle-Haimanot et al., 2006). Dental fluorosis has been reported among 8-51% of children growing up in areas where drinking water contains more than 1 mg/L of F (Diesendorf, 1986, Narwaria and Saksena, 2013), but also in communities exposed to apparently appropriate F concentrations in drinking waters in the tropics (Ibrahim et al., 1995, Akpata et al., 1997, Akpata, 2001). The prevalence of dental fluorosis has increased, even in communities where there is little or no F in water (Soto-Rojas et al., 2004, Vallejos-Sanchez et al., 2006); its prevalence in naturally fluoridated and non-fluoridated communities in Mexico ranged from 30 to 100% and from 20% to 45% respectively. A cross-sectional study (Wong et al., 2014) that compared the prevalence and severity of diffuse opacities among 12 year old Hong Kong Chinese children whose maxillary incisors developed during different periods (1983, 1991, 2001 and 2010) with different concentrations of F (1.0, 0.7, 0.5 and 0.5 ppm respectively) in the public water supply, due to changes in the water fluoridation programme in the city, showed a decrease in prevalence and severity from 1983 and then an increase in 2010. From the marked differences in the prevalence and severity of diffuse opacities among these Hong Kong Chinese children it was observed that the decrease in the

prevalence and severity of diffuse opacities among the maxillary incisor teeth corresponded to the reductions that occurred in the concentration of F in the public water supply (1.0 ppm in 1970, 0.7 ppm in 1978 and 0.5 ppm in 1988), during their enamel development (Wong et al., 2014). However, the increased prevalence and severity of diffuse opacities in 2010 when the F concentration remained the same as in 2001 were suggested to be due to ingestion of F from other sources.

2.9.1.2 Artificial water fluoridation

Water can be artificially fluoridated in non-fluoridated communities where the prevalence and/or incidence of dental caries is high or increasing. Many reports have concluded that water fluoridation is a safe and effective method for preventing dental caries (Doessel, 1985, 2005). The WHO emphasized that water fluoridation benefits all residents served by community water supplies regardless of their social economic status and where it is technically feasible and culturally acceptable has substantial advantages to public health (Petersen and Lennon, 2004). Moreover, water fluoridation has been recognized as one of the 10 greatest public health achievements of the 20th century by the United States Centres for Disease Control and Prevention (Centres for Disease Control and Prevention, 1999). The WHO later adopted it as an effective oral health intervention and at least about 30 nations have instituted artificial water fluoridation policies (Peckham and Awofeso, 2014). As less than 10% of the drinking water sources in Nigeria are from waterworks, artificial fluoridation of pipe-borne water would benefit only a small fraction of the Nigerian community (Akpata et al., 2009).

2.9.1.3 Beverages

Beverages such as tea, fruit juices, soft drinks, carbonated drinks and alcoholic beverages (wine, beer and liquor) have been reported to contain wide variations in F concentration. Tea (*Camellia sinensis*) is naturally rich in fluorine (Han et al., 1995) and other components including aluminium (Al) (Flaten and Lund, 1997). The tea plant absorbs F and Al in considerable amounts from the soil by passive diffusion and accumulates them in the leaves (Ruan and Wong, 2001). The concentration of F in tea varies from one country to the other and it depends on the plant's botanical variety, the soil where it is grown and the procedure of preparation of the tea infusions. Several papers have been published on the F content of tea infusions of different origins and types using a F-ISE based method and shown a range between 2.10–123 mgF/kg in Chinese green (Cao et al., 2000a) and black teas, 35–182 mgF/kg in Iranian black teas, 352-1175 mgF/kg in Chinese brick tea (Cao et al., 2000a),

97–148 mgF/kg in black tea leaves and 139–223 mgF/kg in black tea bags originating from United Kingdom, India, China, Japan, and Sri Lanka, and 311–604 mgF/kg in some black tea brands marketed in the US, UK, China and Hong Kong (Cao et al., 2006). In countries where tea is consumed regularly, after water, tea could potentially be the second most important source of dietary F intake. In countries where tea is culturally consumed regularly, it plays an important role in triggering the undesirable effects of F on tooth formation. The intake of F via tea can be high in areas where water with high F content is used to prepare tea. F intake from tea has been strongly correlated with dental fluorosis in some endemic fluorosis Chinese communities (Han et al., 1995, Cao et al., 1997). A cross-sectional study (Wei et al., 2014) on drinking tea-borne dental fluorosis in Quinghai Province, China reported the occurrence of fluorosis among the different occupational groups who consumed different kinds of tea. Similarly, a laboratory study (Rahim et al., 2014) that measured F concentration in beverages in Malaysia reported a mean F concentration as high as 13.02(±0.23) mg/L in tea packet drink. In addition, the mean F content in both packet and hawkers' drinks were 7.64(1.88) mg/L and 7.51(1.60) mg/L respectively. In Nigeria, the quantity of tea consumed daily has not been determined and anecdotally, the consumption of tea is common among most categories of peoples and households. In Nigeria, tea is consumed during cold weather as hot beverages or as iced tea when the weather is hot. Tea consumption in Nigeria is not only restricted to drinkers' households, it is purchased and drunk at bus-stops, motor garages and it is hawked by different classes of people who earn their living through this means.

Regarding other beverages, in a laboratory study (Maguire et al., 2012) that measure F content of ready-to-feed infant drinks and foods in the UK, the median (range) F content of ready-to-feed juice and milk was 0.069(0.05-0.15) and 0.12(0.01-0.03) μg/ml respectively. Also, a laboratory study (Bhatti et al., 2010) in Kuwait on F concentrations in soft drinks, fruit juices and milk samples using an ISE showed F concentration ranged from 0.05-0.15 mg/L in soft drinks, 0.05-0.20 mg/L in fruit juices and 0.02-1.20 mg/L in milk samples collected from Kuwaiti markets. Soy-based formulas, beverages and juices have been considered as substitutes for breast milk or cow's milk especially in children who have lactose intolerance or cow milk allergy or in cases where breastfeeding is discontinued early for any reason (Rieu, 2006, Turck, 2007). Some studies have reported high levels of F in soy-based products (Silva and Reynolds, 1996, Pagliari et al., 2006) therefore, for younger children and for those with a diagnosis of lactose intolerance or cow's milk allergy, the identification of the most recommended soy-based products is important to

minimize the possible risks of fluorosis occurrence (de Carvalho et al., 2013). Given that the F content is not available on the products' labels, knowing the levels of F in soy-based diets is important. Powdered infant formula and infant formula concentrate are particularly important contributing sources for higher amounts of F. Some brands of these products contain sufficient amounts of F that when mixed with optimally fluoridated water result in greater than optimal amounts of F in the formula (Berg et al., 2011). There is scarcity of data on F concentration in beverages such as tea and ready-to-feed infant drinks in Africa especially sub-Saharan Africa, therefore there is a need for studies that will report F intake from these products.

2.9.1.4 Solid foods

Almost all foodstuffs contain at least traces of F. Virtually all vegetation contains some F which is absorbed from soil and water. Many elements and compounds such as F, copper, zinc, iodine, selenium, cadium, nickel and mercury are found in the soil and they are ingested into the body via the food chain or water through soil leaching (Wang et al., 2014). Excessive or deficient concentrations of these elements and compounds in soil can cause adverse health problems in humans (Wang et al., 2014). Animal and human cross-sectional studies (Yue et al., 2009, Lohakare et al., 2010) have reported interactions between F and other elements which may either potentiate or aggravate the occurrence of dental fluorosis. Estimates of the F concentration in various types of foods vary widely, from 0.003 to 1.500 ug/g in some vegetables (Schamschula et al., 1988b), 0.10 to 1220 ug/g in some fish (Walters et al., 1983), 0.003 to 1.09 ug/g in some fruits, 0.9 to 14.4 ug/g in some spices and pepper and from 0.88 to 17.60 ug/g in some cooking and table salts (Taves, 1983). It is important to mention that variability in the F concentrations in vegetables may not be directly related to the water and soil F concentrations of the geographical locations where the vegetables are grown and may relate more to selective uptake of F by the plants. It has been shown that the F concentration of garden products from high F areas compared to low F areas was not associated with the F concentration of water in their respective areas (Schamschula et al., 1988b).

The F contents of prepared and processed foods are usually higher than raw foods and this may be due to the type of water used in processing them however, the increase in the F content of processed foods is not always proportional to the F concentration of water used in processing them. Also, the use of additives such as salt, spices and pepper and the method of food preparation may increase the F content of foods. Observational studies

(Yoder et al., 1998) carried out in Tanzania have established that magadi, a trona (sodium carbonate and sodium bicarbonate) which is often used as a food tenderizer and for speeding cooking time contains F in varying concentrations depending on the source. A laboratory and observational study (Kaseva, 2006) that investigated the contribution of magadi to the prevalence and severity of fluorosis in northern Tanzania showed that F concentrations in magadi samples were in the range of 0.21 to 0.9 mg/g and high concentrations of F in magadi suggested that excessive fluorosis may be due to the use of magadi in food preparations. The material and composition of a cooking vessel may also influence the F content of the cooked food. Aluminium pots when used reduce the F content of cooked foods because of the formation of aluminium-F (Full and Parkins, 1975, Dash and Sethi, 2012) but steel and Pyrex pots showed no changes (Full and Parkins, 1975). However, Teflon-coated pots released F into foods when used (Full and Parkins, 1975).

2.9.2 Non-dietary sources of systemic F exposure

2.9.2.1 Toothpaste ingestion

Over 95% of commercially available toothpastes contain F compounds as sodium F (NaF), sodium monofluorophosphate (Na_2PO_3F, SMFP), stannous F (SnF_2) or amine F (Hattab, 1989). Most fluoridated toothpastes contain 1000 – 1450 ug/g and at this concentration 1 g of toothpaste contains about 1.0-1.5 mg F (Adair, 1999). Toothpaste is the most widely used form of topical use of fluorides, however in intentional ingestion of fluoridated dentifrice during tooth brushing together with exposure to F from various other sources could result in excessive F ingestion potentiating the risk of dental fluorosis development (de Carvalho et al., 2013). A number of studies have reported that frequent tooth brushing and tooth brushing before 2 years of age were risk indicators for dental fluorosis (Osuji et al., 1988a, Lalumandier, 1992, Vallejos-Sanchez et al., 2006). However, the search to determine optimum F concentration in toothpastes with maximum anti-caries benefit and minimum fluorosis potential should continue (Elkhadem and Wantees, 2014) while improving knowledge of the adverse effects of young children ingesting excessive amounts of F toothpaste. In a systematic review (Wright et al., 2014) of F toothpaste efficacy and safety in children younger than 6 year olds, 17 papers met the criteria for inclusion. The use of F toothpaste led to a statistically significant decrease in mean decayed missing and filled primary tooth surfaces and decayed missing and filled primary teeth for populations at high risk of developing caries. In this review, the effects of using different F concentration

toothpastes on caries varied, with some publications reporting decrease caries prevalence when high or low F containing toothpastes were used. The use of toothpaste after 12 or 14 months of age decreased the risk of fluorosis and ingesting greater than pea-sized amounts could lead to mild fluorosis. To minimize the risk of fluorosis in children while maximizing the caries prevention benefit for all age groups the appropriate amount of F toothpaste should be used by all children regardless of age (Wright et al., 2014). Oral health practitioners should ensure that the appropriate amount of toothpaste is used by counselling caregivers using oral description visual aids and actual demonstration. In addition, the recommendation of low-F toothpaste formulations should be done with caution when considering both risk and benefits because a recent *in-vivo* study of eleven 8-to-10 year old children (Pessan et al., 2014) showed that the use of low-F dentifrice (513 ppmF) did not promote a higher F uptake in inner biofilms sections and plaque F was significantly elevated only after use of the conventional dentifrice (1072 ppmF). In Nigeria, recent studies (Okoye and Ekwueme, 2011, Bashiru and Omotunde, 2014) have reported an increase in tooth brushing with F toothpaste due to increased access to toothpaste and toothbrush as well as increased awareness to good oral hygiene.

2.9.2.2 F containing chewing sticks

Chewing sticks such as miswaks are used in many countries including Nigeria for cleaning purposes, often up to 3 times daily. Studies (Adekola and Akinola, 2001, Fazlul Hoque et al., 2007) have shown that some type of chewing sticks naturally contain F in the range of 50 to 222 ppmF. Some *in-vitro* and *in-vivo* studies (Baeshen et al., 2008, Baeshen and Birkhed, 2010) have also used chewing sticks as a vehicle for delivering F by impregnating them with F. Anecdotally, it has been observed that some children in Nigeria especially in rural communities are provided chewing sticks as tooth cleaning aids by their parents. A Nigerian study (Okoye and Ekwueme, 2011) among 301 eleven to 16 year olds showed that about 12% reported use of chewing sticks.

2.9.2.3 Other non-dietary sources of systemic F exposure

Other non-dietary sources of systemic F intake such as F supplements in form of tablets, drops and lozenges, mouthrinses (Weyant et al., 2013) and gels (Marinho et al., 2003) have been used as substitute for fluoridated water to prevent the occurrence of dental caries especially among high risk children in non-fluoridated areas. However, their intake and use in children should be under close supervision to prevent excessive intake. Unpublished

reports have shown that F supplements, mouthrinses and gels are not used in developing countries like Nigeria.

F can be ingested from air when they are emitted from substances such as coal. F can be found in dust as well as in plants and soil from emissions from F producing chemical plants. Environmental monitoring of F emissions using precipitation, dust, plant and soil samples in Germany showed a pronounced pollution gradient in precipitation, dust, plants and soil samples (Franzaring et al., 2006). Fluorosis has been associated with coal-fired pollution and F-rich coal used as indoor fuel sources in China (Zhang et al., 2014a). Nigeria has a large coal reserves in excess of 1.2 billion tones (Onoduku, 2014) but there is dearth of data on the magnitude of coal burning.

This section has highlighted systemic F exposure from dietary and non-dietary sources and reported variability in the F concentration of these sources as well as the amount of drinks and foods consumed. The water consumed as drinking or cooking water in sub-Saharan Africa like Nigeria is from ground water because of inadequacy of pipe-borne water. Similarly, the use of F supplements, mouthrinses and gels in prevention of dental caries is not popular in the continent of Africa. There is a scarcity of data on F content of drinks and foods in Africa which may translate to lack of information on systemic F intake.

2.9.3 Systemic F intake and its assessment

In the past, water F concentration was a reasonable predictor of F intake and risk of dental fluorosis, however, the increased availability and ingestion of F from other sources e.g. toothpastes, salt, tablets, milk or bottle water and the drying of foods over F rich coal fires (Zohouri and Rugg-Gunn, 2000b, Levy et al., 2001, Whelton et al., 2004, Do and Spencer, 2007, Maguire et al., 2007, Rodrigues et al., 2009) has complemented the effects of water F concentration. For most communities the major sources of F intake are dietary and non-dietary, but to assess fluorosis risk adequately, it is important to consider all potential F sources when estimating F intake. An accurate estimation of F intake is possible but it requires good co-operation from subjects, skilled staff to record intake and can be expensive (Villa et al., 2010). If it were possible to obtain valid predictions of F intake from measurements of urinary F excretion, the risk of dental fluorosis might be assessed more easily since UFE is a contemporary marker and collecting urine from children who are most at risk of dental fluorosis is challenging (Marthaler, 1999, Villa et al., 2000, Franco et al., 2005b). Many studies have assessed and reported F intake from both dietary and non-dietary sources of F in both fluoridated and non-fluoridated areas in different age groups

(Table 2.5). Since it has been estimated that the average daily F intake from air through inhalation would be 0.001 to 0.004 mg (Martin and Jones, 1971), the contribution of this source to TDFI is very low and it is the F content of water, beverages, foods, dentifrices and supplements which primarily determine intake, along with inadvertent F ingestion from toothpaste or in the form of dietary supplements. The most important sources of F for the majority of children are diet and dentifrice.

2.9.4 Assessment of dietary F intake

Assessment and comparison of dietary F intake in infants, children and adults is difficult due to wide variation in the amount and nature of foods eaten by individuals and the eating habits of different societies. In addition, F concentrations of different food items vary and might be due to different F concentrations in waters used to process foods and drinks. The dietary nutrient intakes of individuals can be assessed in 2 major ways namely: (a) prospectively, in which drinks and foods are recorded as consumed and (b) retrospectively, in which drinks and foods consumed previously are recalled.

2.9.4.1 Prospective methods

The main dietary assessment methods used to investigate dietary intakes of individuals prospectively are food records and duplicate methods.

Food record method

In a food record method, the details of food and beverages consumed together with brand name, cooking and preparation methods, ingredients of mixed dishes and time of consumption is recorded by the respondent in a diary. The time period used is usually 3, 5 or 7 consecutive days (Thompson and Byers, 1994) however, it has been shown that recording for more than 4 consecutive days may result in a decrease in reported intakes (Gersovitz et al., 1978). To validate other methods, a 7-day food record has been suggested as the gold standard (Willet, 1998) however, a decrease in validity of the collected information has been reported when the number of days increased. Assessment of dietary intakes in the UK in national surveys such as the National Diet and Nutrition Survey (NDNS) using a 7-day weighted dietary record have provided a comprehensive, cross-sectional picture of the dietary habits of 1701 children aged 4-18 years (Gregory et al., 2000). In Germany, a 3-day weighed food record was employed in a longitudinal study to assess the protein intake of 439 children and adolescents (Bokhof et al., 2010). In a study (Schamschula et al., 1988b) on daily F intake from the diet of Hungarian children, the F

intake of the children was assessed using a 7-day food record. A 3-day food diary with a face-to-face interview on the 4th day was used to assess dietary F intake of children in Iran (Zohouri and Rugg-Gunn, 2000b) and England (Maguire et al., 2007).

In the food record method, portion sizes of food and drinks are weighed either by a scale or household measures such as cups and tablespoons or estimated using models or pictures. This method allow the sources of food to be identified, dietary habits are not altered and records are made at the time of consumption, avoiding omission of food and drinks consumed. However, this method has some limitations such as being burdensome to respondent and researcher since respondents need to record detailed description of drinks and foods and the researcher needs to check records for completeness and missing entries. The checking of the records should be done with the respondents using 2 or 3 dimensional food models to aid respondents to quantify food portions (Brunner et al., 2007). This method requires a food composition table, however, there is no comprehensive food composition table for F. In addition, this method requires that the respondent should be literate, motivated and fully-cooperative thus its use among illiterate populations is limited making it subject to bias both in sample selection and dietary measurement (Thompson and Subar, 2001). Other errors such as coding errors and incorrect recoding of foods and drinks consumed can also occur (Anderson, 1995).

Duplicate Diet method

A Duplicate Diet method involves retaining an identical portion of all foods and drinks consumed throughout the day by subjects. These identical foods and drinks are then weighed and analysed by the researchers. In this method, food consumption tables are not used because the actual foods or drinks consumed by the participants are analysed therefore it is an objective method of dietary assessment. Furthermore, this method is not subject to inaccurate recording of the amounts, inadequate coding and non-inclusion of a given food type in the food consumption tables. Studies on assessment of nutrient intakes among children (Goshima et al., 2008, Sugiyama et al., 2009) and adults (Kim et al., 1984, Bro et al., 1990) have utilized this method. Similarly some studies used the duplicate method to measure F intake in children (Franco et al., 2005b, Nohno et al., 2006). The method is regarded as one of the most accurate ways of sampling the diet since the diet is duplicated (Guha-Chowdhury et al., 1996). However, this method has some limitations such as the cost of duplicating the diet and is therefore unsuitable for large-scale studies. In addition, the burden on participants to provide the duplicated food and drink can lead to participants

altering their dietary habits to ease the burden. Another limitation of this method is the inability of sources of nutrient intake to be identified since food and drinks are pooled.

Retrospective methods

Retrospective methods involve the collection of dietary data over a period of 24-hour, week or year and are designed for large scale epidemiological studies due to their lower costs. In addition, they are easier to administer and present minimum burden for participants. However, some information is likely to be omitted since they rely on memory recall. These methods are also unsuitable for populations such as children and elderly who have difficulties with memory. The main dietary assessment methods used to investigate dietary intake of individuals retrospectively are 24-hour recall, market basket collection, food frequency questionnaires (FFQs) and diet history.

24 hour recall method

The 24-hour recall method involves a short interview of study participants by a trained data collector preferably a trained nutritionist or dietician via telephone, computer-assisted programme or paper records. In this method, the participant is asked to list all the drinks and foods consumed during the previous day. The method provides detailed descriptions of the food and drink namely: brand names, composition and preparation of the drinks and foods as well as the portion size, therefore, the complete nutrient intake can be calculated (McPherson et al., 2000). It is quick, simple and inexpensive thus suitable for clinical dietetic studies. It also has a higher response rate than other retrospective methods, although it requires a trained nutritionist or dietician to interview participants, assess portion sizes and make appropriate enquiries about type of foods and drinks consumed as well as those which might be omitted such as snacks. Multiple records rather than a single 24-hour recall should be collected to accurately estimate nutrient intake due to day-to-day intra-individual variation in food intake (McPherson et al., 2000). However, in studies with children, multiple 24-hour records put a lot of burden on parents or caregivers since they need to provide this information over a period of time and this method might be subject to bias in recalling the food and estimating portion sizes.

Market basket collection

The market basket collection involves collection of representative composite food and drink groups found in the diet of study participants according to shopping guidelines so as to estimate the dietary intake of certain nutrients. The guideline which shows the actual 14-28

days consumption of various dietary components consumed by participants is derived from a household consumption survey. The amount of the target nutrient is then determined in each composite food and drink groups using analytical methods. One limitation of this method of assessing dietary intake is that it might not be accurate since the type and amount of food consumed by children is not considered separately in the household survey. In addition, the method places huge burden on the researchers if the household food consumption has not been conducted since it has to be conducted at least 14 days prior to analysis. However, this method has been widely used to investigate the level of some nutrients or pesticides intake from food in large epidemiological studies (Darnerud et al., 2006, Schecter et al., 2006). The method has also been used to assess dietary F intake of infants and young children in the USA (Ophaug et al., 1980b, Ophaug et al., 1985).

Diet history

A diet history quantitatively measures individual's habitual dietary intake over a specified period of time thereby determining actual food intake of the preceding day. This information is collected during an interview and is cross checked with the food groups. This interview requires a skilled person and could take up to an hour. This method does not require the respondent to be literate and does not alter dietary habits. It assesses meal patterns and details of food and drink intakes rather than intakes for a short period (Thompson and Subar, 2001). A dietary history was found to be more representative of habitual intake when compared to diet record (Livingstone et al., 1992). The limitation of this method is that the recall may not be precise and it requires a highly trained interviewer. This method has been used in several studies in adults (Van Staveren et al., 1985, Visser et al., 1995, Black et al., 2000). However, with children it is difficult to obtain the information therefore, requiring parental input.

Food Frequency Questionnaire (FFQ)

Food frequency questionnaires (FFQs) are used to measure food and drink intakes of individuals over a specific period of time usually 6 months to one year. The questionnaire contains list of specific food and drink items and a section for reporting how often each food and drink is consumed. In addition, semi-quantitative FFQs ask respondents to provide information on portion size by asking respondents to select portion size from atlas of food and drink portion or standard portion size described on the questionnaire (Nelson et al., 2007). Nutrient intakes are then determined by multiplying food frequency scores for individual food and drink items by the nutrient content of the local standard portion or

estimated portion size (Bingham et al., 1988). Some factors such as length of the questionnaire, type of response (open or closed-ended), portion size inclusion, seasonality and time frame over which respondents should recall the food and drink they consumed should be considered carefully in the design of the FFQ. Some FFQs are designed without reporting portion sizes of foods and drinks because the frequency of consumption is a greater contributor to intake than serving size (Heady, 1961, Willet, 1998). Inclusion of questions about portion size and frequency of consumption depends on the study objectives and population characteristics (Willet, 1998). Some studies have used the FFQs to assess dietary intake among children (Blum et al., 1990, Marshall et al., 2003). Similarly, FFQs have been used to investigate the relationship between dietary intake and diseases among adults (Porrini et al., 1995, Pisani et al., 1997). Dietary F intake among children in Iowa has been investigated in a longitudinal study using the FFQ (Levy et al., 2001, Levy, 2003). Similarly, a semi-quantitative FFQ was used in Brazil to determine dietary F intake of 2 to 6 year old children. One of the advantages of the FFQs is that they provide practical and cost-effective way of collecting dietary information from large number of respondents over a prolonged period. However, there are disadvantages in using the FFQ such as collection of few details about the characteristics of foods and drinks consumed and it can contain a significant amount of measurement error.

In summary, dietary F intake can be assessed by recording the diets as they are consumed prospectively and by recalling how they were consumed retrospectively. Recording the diets as they are consumed allow the details of the diets to be recorded however, it is costly, places burden on the respondent and researcher and require that the respondent be literate, motivated and fully-cooperative. Recalling how diets were consumed such as using FFQ are designed for large epidemiological studies like this present study due to their low cost. Furthermore, the use of FFQ present minimum burden for the participants since it is easy to administer, however, it subject to recall bias. Globally, the various methods of assessing dietary F intake have been used in several epidemiological studies among various population groups.

2.9.5 Studies which have assessed dietary F intake (DFI)

Table 2.5 shows a summary of studies that have assessed dietary F intake among children based on country, region, age, method of dietary F intake assessment and community water F. The majority of the studies were undertaken in South America while few were undertaken in Africa. Dental fluorosis is a major public health problem in both regions but

the reason for the difference in the amount of studies in these two regions might be due to more availability of funds and other research supports in South American than in Africa. Dietary F intake studies require large amounts of funds to undertake both field and laboratory work. It was surprising to observe that only one study was undertaken in Asia despite high prevalence of dental fluorosis in some regions in Asia. Many studies in South America also reported F intake from toothpaste and total daily F intake while the studies in Africa only reported dietary F intake. Reporting F intake from diet and toothpaste allows TDFI to be calculated. It also allow their contribution to TDFI to be calculated. The majority of the dietary F studies were undertaken among young children between ages 0.1 month to 7 years because the window of susceptibility of the teeth to dental fluorosis occur during this age range. Many studies used the duplicate diet method to assess dietary F intake probably due to its reported high degree of accuracy but it is unsuitable for large scale studies because of its cost. Dietary F intake studies that recruited many participants used the FFQ because it is a cost effective way of collecting dietary information from a large number of respondents over a prolonged period.

The contribution of dietary sources to F intake varies among different populations depending on amount and F concentration of water consumed, age of the individuals, climate and dietary practices (Murray, 1986). In Table 2.5, daily F intake in fluoridated areas varies from 0.31 mg/day among 1-3-year-old Brazilian children (De Almeida et al., 2007) to 14.5 mg/day among 1-4-year-old Kenyan children (Opinya et al., 1991a) while in non-fluoridated areas it varies from 0.15 mg/day among 3-4-year-old New Zealand children (Guha-Chowdhury et al., 1996) to 0.41 mg/day 4-year-old Iranian children (Zohouri and Rugg-Gunn, 2000a). Based on weight, it ranged from 0.01mg/kg bw/day among less than 4-year-old Brazilian children (Zohoori et al., 2013a) to 0.05 mg/kg bw/day among 3-4-year-old Palestinian children living in fluoridated areas (Abuhaloob et al., 2015) while it was 0.01 mg/kg bw/day among 6-7-year-old UK (Maguire et al., 2007) and 0.03 mg/kg bw/day among 4 year old Iranian children living in non-fluoridated areas (Zohouri and Rugg-Gunn, 2000b). The only study on F intake in Nigeria was a cross-sectional study in the south of the country that determined F ingestion from drinking water consumed by 314 one to three year Nigerian children (<10-year-old) exposed to 0.7 ppm water which recorded a mean intake of 0.062 (0.023) mg/kg bw/day (Akpata, 2004a).The differences in dietary F intake in these studies might be due to differences in F concentration in water, geographical location, age group studied and method used for assessing dietary F intake.

Water is used for drinking or added to other drinks and foods during preparation and cooking therefore it can be a major source of F intake in some children living in fluoridated areas especially if the F content in water is high. The F concentrations of drinks and foods vary considerably due to variation in the concentration of F in water as well as the amount of water used for preparation and or cooking (Clovis and Hargreaves, 1986). The contribution of drinking water to total dietary F intake was 63% in 8-9-year-old children living in fluoridated areas of Mexico (Grijalva-Haro et al., 2001) and between 11 to 24% in 6-7-year old British children living in optimally and sub-optimally fluoridated areas (Zohouri et al., 2006a). Drinking water was shown to be the main dietary contributor of F in artificially and naturally fluoridated areas in a cross-sectional study on dietary F intake by 4-6-year-old Brazilian children receiving different sources of systemic F (Rodrigues et al., 2009). Approximately 50-75% of dietary F intake in young children is derived from drinks comprising water and beverages (Zohouri and Rugg-Gunn, 2000b). The contribution of drinks to dietary F intake varies from 7% in 6-7-year-old British children living in non-fluoridated water areas (Maguire et al., 2007) to 72% in 3-6-year-old German children living in salt fluoridated areas (Haftenberger et al., 2001). The observed differences in the percentage contribution of drinks to dietary F intake in these studies might be due to differences in the F concentration and amount of water and drinks consumed as well as age differences.

The contribution of food to dietary F intake was 27% in 16-40-months-old US children (Rojas-Sanchez et al., 1999), 60%-67% in 3-4-year-old Palestinian children (Abuhaloob et al., 2015) and 84% in 15-36-months-old Mexican children (Martínez-Mier et al., 2003). Foods cooked with water such as rice and pasta have been reported to contribute substantially to dietary F intake (Zohouri et al., 2006a). This was also demonstrated in two cross-sectional studies that reported high dietary F intake ranging from 1.11mg/day in 4-year-old Hungarian children (Schamschula et al., 1988b) to 5.41mg/day in 8-9-year-old Mexican children (Grijalva-Haro et al., 2001) living in areas with high F concentration in drinking water. The differences in the percentage contribution of food to dietary F intake in these studies might be due to different F concentration of water used to prepare or cook the foods, dietary practices and methods used to determine dietary F intake.

2.9.6 Assessment of F intake from toothpaste

It is generally accepted that the most important factor in the decline in dental caries is the widespread and early use of F toothpaste (Bratthall et al., 1996). The widespread and early

use of toothpaste might be due to worldwide availability of low cost toothpaste. However, the early use of F toothpaste is one of the factors that may be associated with an increased risk of dental fluorosis in both fluoridated and non-fluoridated communities (Osuji et al., 1988a, Lalumandier and Rozier, 1995, Ellwood and O'Mullane, 1995). This is because fluoridated toothpaste can be inadvertently ingested and could be a major source of F intake in some children since all the F swallowed is readily bioavailable and absorption is close to 100% (Ekstrand and Ehrnebo, 1980). Therefore, ingestion of F from swallowed fluoridated toothpastes during the critical period of tooth development (birth to 6 years) can be a risk factor for dental fluorosis (Osuji et al., 1988a, Lalumandier and Rozier, 1995, Mascarenhas and Burt, 1998, Pereira et al., 2000). This is because the developing tooth bud is exposed to high F concentrations from the ingested toothpaste and the risk is particularly marked if a child chronically swallows toothpaste, especially adult toothpaste. The permanent dentition is at risk of dental fluorosis during the first 7 years of life (Ishii and Suckling, 1991) but reports have shown that the aesthetically important permanent maxillary incisors are most susceptible before 2 years of age (Evans and Stamm, 1991, Van Palenstein Helderman et al., 1997). Barsden and co-workers (Bårdsen et al., 1999) reported that the susceptibility of the permanent incisors and the first permanent molars to fluorosis would appear to be greatest during the first 4 years of life since these teeth begin to form soon after birth and erupt at about 7 years of age. However, chronic excessive ingestion of systemic F from 3 to 6 years of age can also cause dental fluorosis in the permanent canines, premolars and second molars that erupt later (Levy, 2003) since these teeth are formed and calcified during this period.

Several factors have been reported to enhance the likelihood of fluoridated toothpaste being ingested namely; inability to control swallowing reflexes, early start of brushing with fluoridated toothpaste before age of 2 years (Osuji et al., 1988a), unsupervised tooth brushing (Levy and Zarei-M, 1991) and use of large amounts of toothpaste (Levy et al., 1993, Moraes et al., 2007). Swallowing of toothpaste by children during tooth brushing occurs due to the pleasant flavouring agent which could stimulate them to swallow the toothpaste loaded onto their toothbrush (Moraes et al., 2007). Other brushing habits such as frequency of brushing (Pendrys, 1995) and the F concentration of toothpastes (Rock, 1994) have been shown to contribute to greater F intake from toothpastes and subsequently the risk of developing dental fluorosis. Similarly some observational studies (Naccache et al., 1992, Sjögren et al., 1994) reported that brushing activities including rinsing and expectoration have been reported to be associated with F intake from toothpaste.

Fluoride intake from toothpaste can be assessed by two methods. One method measures the proportion of F retained on the toothbrush and in the expectorate including any after-brushing rinses during (Maguire et al., 2007, Zohoori et al., 2012). Measuring F concentration in after brushing rinses can help to determine the importance of rinsing and spitting on F ingestion from toothpaste. Fluoride ingestion from toothpaste is significantly reduced by rinsing and/or spitting (Van Loveren et al., 2004). The other method measure the amount of F toothpaste used based on parents' questionnaire responses to a series of diagrams of toothbrushes holding varying amounts of toothpaste with parent's selecting the diagram that best depicted the amount that the child routinely uses and estimating the amount ingested based on the amount retained on the brush and in any expectorate (Franzman et al., 2006). This individual level information is useful for informing epidemiological studies involving large number of people where individual tooth brushing behaviours cannot be observed directly and where estimates need to be used.

2.9.7 Studies which have assessed F intake from toothpaste ingestion

Some observational studies that have assessed actual F intake from toothpaste by measuring the proportion of F that are retained on the toothbrush and in the expectorate have reported the amount of toothpaste swallowed by children. Information on F intake from toothpaste especially in fluoridated areas is important because of the complementary effect of F from both toothpaste and water on the development of dental fluorosis. It is also important in determining the optimal F intake that provides maximum caries benefit and minimal dental fluorosis. Table 2.5 shows the F intake from toothpaste and percentage contribution of toothpaste to total daily F intake by country, age and F exposure. In fluoridated areas, the F intake from toothpaste varies from 0.20mg/day among 0.1-1-year-old UK (Zohoori et al., 2014) to 1.34mg/day among 1-3-year-old Brazilian (De Almeida et al., 2007) children while in non-fluoridated areas, it varies from 0.10mg/day among 0.1-1-year-old UK (Zohoori et al., 2014) and 1.21mg/day among 3-4-year-old New Zealand (Guha-Chowdhury et al., 1996) children.

Authors (Year)	Country	n	Age (years)	Food & Drink collection method	Fluoridated areas			Non-fluoridated areas		
					Diet[a,b] & % contribution to TDFI	Toothpaste [a, b] & % contribution to TDFI	TDFI [a, b, c]	Diet[a,b] & % contribution to TDFI	Toothpaste [a, b] & % contribution to TDFI	TDFI [a, b, c]
Europe										
Schamschula et al. (1988b)	Hungary	18 28 21	3.9	7 day food record	NR 0.72[a] 1.11[a]	NR NR NR	NR NR NR	0.22[a] NR NR	NR NR NR	NR NR NR
Zohouri et al. (2006a)	UK	33	6-7	3-day diet diary	Drinks 55%-59%	NR	NR	Drinks 32%	NR	NR
Maguire et al. (2007)	UK	33	6-7	3-day diary	0.016-0.025[b] 53-65%	0.022[b] 35-47%	0.038-0.047[b]	0.008[b] 43%	0.023[b] 57%	0.031[b]
Zohoori et al. (2013b)	UK	33	6-7	Duplicate diet	NR	NR	0.076[b]	NR	NR	0.038[b]
Zohoori et al. (2014)	UK	38	0.1-1	3-day diet diary	NR	0.20-0.50[a]	0.107[b]	NR	0.10-0.50[a]	0.024[b]
North America										
McClure (1943)	USA	NR	1-3	NR	0.42-0.83[a]	NR	0.026-0.103[b]	NR	NR	NR
Ophaug et al. (1985)	USA	44	0.6 2	Market basket	0.42[a] 0.62[a]	NR NR	0.05[b] 0.05[b]	NR NR	NR NR	NR NR
Burt (1992)	USA	NR	1-3 3-6	NR	0.65[a] 0.90[a]	NR NR	0.04-0.07[b] 0.03-0.05[b]	NR NR	NR NR	NR NR
Rojas-Sanchez et al. (1999)	USA	29 11 14	1.3-3.3	Duplicate diet	0.542[a] NR NR	0.424[a] NR NR	0.07[b] NR NR	NR 0.219[a] 0.389[a]	NR 0.548[a] 0.576[a]	NR 0.056[b] 0.073[b]

Authors (Year)	Country	n	Age (years)	Food & Drink collection method	Fluoridated areas			Non-fluoridated areas		
					Diet[a,b] & % contribution to TDFI	Toothpaste [a, b] & % contribution to TDFI	TDFI [a, b, c]	Diet[a,b] & % contribution to TDFI	Toothpaste [a, b] & % contribution to TDFI	TDFI [a, b, c]
Martinez-Mier et al. (2009)	USA	12	1.2-2.5	Duplicate diet	0.55[a]	NR	NR	NR	NR	NR
South America										
Villa et al. (2000)	Chile	20	3-5	2-day duplicate diet	0.765[a] 75.4%	0.254[a] 24.6%	0.064[b]	NR	NR	NR
Grijalva-Haro et al. (2001)	Mexico	20	8-9	2-day duplicate diet	2.31[a]	NR	NR	NR	NR	NR
Lima and Cury (2001)	Brazil	39	1.8-2.6	2-day Duplicate diet	45%	55%	0.090[b]	NR	NR	NR
Paiva et al. (2003)	Brazil	71	1.7-3.2	2-day duplicate	0.027-0.040[b]	0.052-0.061[b]	0.088-0.090[b]	NR	NR	NR
Martínez-Mier et al. (2003)	Mexico	46	1.3-3	Duplicate diet	28%-36%	64%-72%	0.18-0.20[b]	NR	NR	NR
Franco et al. (2005b)	Colombia	120	4-5	Duplicate diet	34%	66%	0.098[b]	NR	NR	NR
Franco et al. (2005a)	Colombia	118	1.1-2.1	Duplicate diet	0.040[b]	0.107[b]	0.147[b]	0.41[a] 30%	0.97[a] 70%	1.38[a] 0.07[b]
De Almeida et al. (2007)	Brazil	33	1-3	Duplicate diet	0.31[a] 0.025[b]	1.34[a] 0.106[b]	0.130[b]	NR	NR	NR
Martins et al. (2008)	Brazil	29 20	1.7 3.3	NR	0.031[b] 0.029[b]	0.051[b] 0.049[b]	0.083[b] 0.084[b]	NR	NR	NR

Authors (Year)	Country	n	Age (years)	Food & Drink collection method	Fluoridated areas			Non-fluoridated areas		
					Diet[a,b] & % contribution to TDFI	Toothpaste [a, b] & % contribution to TDFI	TDFI [a, b, c]	Diet[a,b] & % contribution to TDFI	Toothpaste [a, b] & % contribution to TDFI	TDFI [a, b, c]
Miziara et al. (2009)	Brazil	379	2-6	FFQ	0.478[a] 0.027[b]	0.614[a] 0.036[b]	1.092[a] 0.064[b]	NR	NR	NR
Rodrigues et al. (2009)	Brazil	121	4-6	2-day duplicate diet	NR	NR	0.04-0.06[b] (water, salt, milk)	NR	NR	0.01[b]
Rodrigues et al. (2009)	Brazil Brazil Lima Peru Trijillo Peru	25 21 26 25	4-6	Duplicate diet	0.33[a] 0.24[a] 0.75[a] 0.63[a]	NR	NR	NR	NR	NR
Levy et al. (2013)	Brazil	398	2-6	FFQ	NR	NR	NR	0.17[a] 51.5%	NR	0.017[b]
Zohoori et al. (2013a)	Brazil	14 15	<4	2-day duplicate diet	0.011-0.015[b]	0.037[b]	0.015-0.048[b]	0.006-0.011[b]	0.055[b]	0.011-0.061[b]
Asia										
Murakami et al. (2002)	Japan	94	3-5	3-day duplicate	NR	NR	NR	0.28-0.30[a]	NR	0.35[a] 0.021[b]
Australasia										
Guha-Chowdhury et al. (1996)	New Zealand	66	3-4	3-day Duplicate diet	0.36[a] 0.019[b]	0.26-1.31[a]	0.68[a] 0.036[b]	0.15[a] 0.008[b]	0.17-1.21[a]	0.49[a] 0.027[b]

Authors (Year)	Country	n	Age (years)	Food & Drink collection method	Fluoridated areas			Non-fluoridated areas		
					Diet[a,b] & % contribution to TDFI	Toothpaste [a, b] & % contribution to TDFI	TDFI [a, b, c]	Diet[a,b] & % contribution to TDFI	Toothpaste [a, b] & % contribution to TDFI	TDFI [a, b, c]
Middle East										
Zohouri and Rugg-Gunn (2000b)	Iran	78	4	3-day diet diary	0.68[a]	NR	NR	0.390[a] 0.028[b]	0.058[a] 0.0039[b]	0.426[a] 0.032[b]
Zohouri and Rugg-Gunn (2000a)	Iran	100	4	3-day diet diary	0.59[a]	NR	NR	0.413[a]	NR	698-3472[c]
Akpata et al. (2014)	Kuwait	400	1-9	FFQ	Drinks only 0.013-0.018[b]	NR	NR	NR	NR	NR
Abuhaloob et al. (2015)	Palestine	216	3-4	3-day diary	0.04-0.05[b] 98.78-99.02%	0.01[b] 0.71-1.22%	0.04-0.05[b]	0.02[b] 99.96%	0.00 0.04%	0.02[b]
Africa										
Opinya et al. (1991a)	Kenya	NR	1-4	NR	14.5[a]	NR	NR	NR	NR	NR
Malde et al. (2003)	Ethiopia	30	<5	4-day duplicate	1.2-8.8[a] - Beverages	NR	NR	NR	NR	NR
Malde et al. (2004)	Ethiopia	30	<5	4-day duplicate	2.3-4.8[a]	NR	NR	NR	NR	NR

Table 2-5: Studies of F intake from diet, toothpaste and total daily F intake of children residing in F and non-F areas.

Notes: [a] – mg/day; [b] – mg/kg bw/day; c - µg/day; NR – Not reported; % - Percentage

Based on body weight, in fluoridated areas, the F intake from toothpaste is reported to range from 0.02 mg/kg bw/day to 0.1 1mg/kg bw/day for UK 6-7-year-olds (Maguire et al., 2007) and 1-3-year-old Brazilians (De Almeida et al., 2007) respectively. In non-fluoridated areas, it has been reported to range from 0.02 mg/kg bw/day to 0.06 mg/kg bw/day for UK 6-7-year-olds(Maguire et al., 2007) and <4-year-old Brazilians (Zohoori et al., 2013a) respectively. A study that investigated F intake from toothpaste by socio-economic group among 1.1 to 2.1 year-old Colombian children reported 0.11 mg/kg bw/day and 0.05 mg/kg bw/day for low and high socio-economic children respectively (Franco et al., 2005a). In these F intake from toothpaste studies, the slightly increased F intake from toothpaste seen in fluoridated areas compared to non-fluoridated areas could increase the risk of fluorosis in the Compared with non F areas. In addition, the observed differences in the F intake from toothpaste in these studies might be due to differences in the age group studied, F concentration and amount of toothpaste used.

Some studies have investigated F exposure from ingested toothpaste by estimating the proportion of toothpaste dispensed which was ingested; 32.9% of dispensed paste was ingested by 4-5-year-old Malaysians (Siew Tan and Razak, 2005), 65% by 2-7-year-old Canadians (Naccache et al., 1992), 60% by 2-3-year old Brazilians (Moraes et al., 2007) and 72% by 30-month-old UK children (Bentley et al., 1999). The contribution of fluoridated toothpaste to TDFI was between 0.7% to 1.2% in 3-4-year-old Palestinian children, very few of whom actually underwent tooth brushing as part of their normal routine (Abuhaloob et al., 2015), while it was 25% in 4-year-old Iranian children (Zohouri and Rugg-Gunn, 2000b) and 72% in both US 15-39 month-olds(Rojas-Sanchez et al., 1999) and 15-36 month-old Mexican children (Martínez-Mier et al., 2003). The differences in the proportion of toothpaste ingested and the percentage contribution of F toothpaste to total daily F intake might be due to differences in the swallowing reflexes of the children studied and the composition of the toothpaste used. Younger children tend to have poor control of swallowing compared with older children and toothpastes with pleasant flavouring agents encourage both intentional and inadvertent swallowing of toothpastes in children.

Inadvertent ingestion of F from toothpaste is common in children and contributes a significant component of F intake. A number of studies have assessed the mean F intake from toothpaste and its contribution to total daily F intake among various groups of children in different countries. However, there is dearth of literature on F intake from toothpaste and percentage contribution of toothpaste to total daily F intake in sub-Saharan Africa.

2.9.8 Assessment of Total daily F intake (TDFI)

Traditionally, the estimation of the mean TDFI has been achieved through the calculation of F ingested from dietary and non-dietary sources in mg/day or mg/kg bw/day in both fluoridated and non-fluoridated areas among different age groups. This is undertaken by adding the F intake from drinks including water, foods and toothpaste in mg/day or mg/kg bw/day. Estimating the mean total daily F intake from drink, food and toothpaste has helped to calculate and compare their contributions to TDFI in the various epidemiological studies.

2.9.9 Studies which have assessed total daily F intake

Total daily F Intake in children has been reported from studies conducted in fluoridated and non-fluoridated areas in various countries. Table 2.5 shows the TDFI of children residing in fluoridated and non-fluoridated areas by country, age, method of food and drink collection and F exposure in water. In Africa, there is dearth of studies on TDFI, the few studies (Opinya et al., 1991b, Malde et al., 2003, Malde et al., 2004, Akpata, 2004a) on F intake among children only reported F intake from drinks and foods. There is a wide variation in the contribution to F intake made from both dietary and non-dietary sources in non-fluoridated and fluoridated areas. In fluoridated areas, the TDFI ranged from 0.02 mg/kg bw/day among Brazilian children aged <4-years (Zohoori et al., 2013a) to 0.107 mg/kg bw/day among UK 0.1-1-year-olds (Zohoori et al., 2014) while in non-fluoridated areas, the TDFI ranged from 0.01mg/kg bw/day among 4-6-year-old Brazilians (Rodrigues et al., 2009) to 0.073 mg/kg bw/day among US 1.3-3.3-year-olds (Rojas-Sanchez et al., 1999). As expected the TDFI reported for fluoridated areas was higher than in non-fluoridated areas thereby showing the influence of water F concentration on TDFI. This influence was further shown in a study (Abuhaloob and Abed, 2013) which explored the association of F concentration in home tap water and total daily F intake (TDFI) among 3-4 year-old Palestinian children and found that the mean TDFI from all sources (drinking water, foods, other beverages and tooth brushing) increased as the F concentration of home tap water increased. The relationship between socio-economic status and TDFI was investigated in a study (Franco et al., 2005b) among 22-35-month-old children in 4 Colombian locations and it was observed that children from low socio-economic status had higher F intake (0.14 mg per kg bw per day) when compared to children from high socio-economic status (0.07 mg per kg bw per day) which was most likely due to a higher consumption of F from food and toothpaste in the lower than higher socio-economic groups.

As Table 2-5 shows, the percentage contribution of diet and toothpaste to TDFI varies widely; the %contribution of diet to TDFI in fluoridated areas ranged from 28% to 98.8% among 1.3-3 year-old Mexican (Martínez-Mier et al., 2003) and 3-4 year-old Palestinian (Abuhaloob and Abed, 2013) children respectively while in non-fluoridated areas it ranged from 30% to 99.9% among 1.1-2.1 year-old Colombian (Franco et al., 2005b) and 3-4 year-old Palestinian (Abuhaloob and Abed, 2013) children respectively.

These differences in overall TDFI and percentage contributions of diets and toothpaste reported in these studies are most likely due to differences in dietary and tooth brushing practices of the study participants, methods used for assessing dietary intake and the age group investigated.

2.9.10 Optimum levels of F intake

It is difficult to assess the threshold level of optimal F intake however, it has been suggested that children should not consume more than 0.10 mg F per kg bw per day to avoid an undesirable degree of dental fluorosis (American Academy Pediatrics Committe on Nutrition, 1986). A study on ingestion of F and dental caries among children aged 1-12-years reported that those living in fluoridated areas with 1.0 µg per ml (1ppm) F in drinking water received between 0.02-0.10 mg F per kg bw per day from all sources including foods (McClure, 1943). McClure (1943) reported that on average the F intake was between 0.05-0.07 mg F per kg bw per day and rarely exceeded 0.10 per kg bw per day. Ophaug and co-workers (Ophaug et al., 1980a) estimated F intake of 6-month-old infants and interpreted the value reported by McClure as a recommendation. Burt (Burt, 1992) in a review of the changing patterns of systemic F intake suggested 0.05-0.07 mg F per kg bw per day as a useful uppermost limit of F intake from all sources such as foods, beverages, dentifrices, mouthrinses, gels and F supplements. The Committee on Medical Aspects of Food Policy, UK recommended F intake of 0.05 mg per kg bw per day as the Upper Limit which is the tolerable upper intake level for infants and young children (Department of Health, 1991). Fejerskov et al. (1987) suggested thresholds of 0.03-0.10 mg F per kg bw per day and dental fluorosis may occur if intakes is more than this limit. Fluorosis has been reported in Kenya at daily average F intake of 0.04 mg F per kg bw per day (Baelum et al., 1987) and other factors such as altitude and genetics might be responsible for the occurrence of fluorosis at a low level of ingestion. A total F intake of 0.05-0.07 mg/kg bw/day in children younger than 12 years of age is regarded as optimum for dental health benefits (Institute of Medicine, 1999). The Upper Tolerable Intake Level of F for children younger than 9-years

is 0.1mg/kg bw/day during infancy to minimise the risk of dental fluorosis (European Food Safety Authority, 2005). The appropriate concentration of F in drinking water as recommended by the WHO Expert Committee on Oral Health and F is 0.5-1.0 ppm which is dependent on climatic conditions and F ingestion from other sources (WHO, 1994a). This expert committee also set the upper limit of F concentration in drinking water at 1.5 ppm.

In summary, the most common source of F is from diets and toothpaste with water contributing greatly to the F ingestion from diet since water is used for drinking and cooking. However, it is mainly ground water from wells and bore-holes, rather than pipe-borne water, which is consumed in many communities in sub-Saharan Africa. Very few studies have reported on F intake from drinks, foods and toothpastes and their contribution to TDFI in Africa, especially sub-Saharan Africa and even fewer have estimated F body burden based on the balance between the amount of F ingested and excreted.

2.10 Fluoride excretion

Fluoride can be excreted in faeces (Falcão et al., 2013), breast milk (Campus et al., 2014), saliva (Petersen et al., 2002), sweat (Brouwer et al., 1988), tears (RCP, 1976) and urine (Maguire and Zohoori, 2013, Akpata et al., 2014), however, F is mainly excreted through the urine.. In general about 5-10% of ingested F may be found in the faeces (Ekstrand et al., 1984) thus, it is assumed that a constant average F fraction of 10% is excreted through faeces (Villa et al., 2000). F excreted in breast milk is usually very negligible, Ekstrand et al.,(Ekstrand et al., 1981) in their report of transfer of F from plasma to breast milk reported that the transfer is very limited. Some reports on sweat as a medium of F excretion have shown that excretion through sweat is small (Henschler et al., 1975, Whitford, 1996b) while some studies have shown high concentration of F in sweat (McClure, 1943, Crosby and Shepard, 1957). Brouwer et al. (1988) suggested that in a tropical climate or during heavy and prolonged exercise, the loss of F with sweat might be significant.

2.10.1 Fluoride excretion through urine

Kidneys are the main route for F excretion from the body and therefore the majority of studies concentrate on estimating urinary F excretion rather than faecal excretion which accounts for only about 10% of F excretion. After ionic F enters the renal tubules, about 10-90% of the ion is reabsorbed and returned to the systemic circulation (Ekstrand, 1996). The absorption of F ions is affected by urinary flow and pH of the tubular fluid. If the tubular

fluid is acidic, more F ions are converted to HF which is diffusible across the tubular epithelium while if it is alkaline nearly all the F will exist in the ionic form and will remain within the tubules to be excreted (Whitford, 1990). Some factors such as diet, altitude and respiratory or metabolic diseases might influence urinary pH (Whitford, 1990). A diet based on meat promoted more acidic urine while vegetarian diet rendered urinary pH alkaline (Ekstrand et al., 1982). Urinary F excretion can also be related to age, climate, total fluids intake and previous exposure to F. Due to difficulties in measuring F intake, urinary F excretion has been suggested as a useful indication of contemporary F exposure. A proportion of ingested F is excreted in urine and plots of daily urinary F excretion against total daily F intake suggest that daily urinary F excretion is suitable for predicting F intake for groups of people but not for individuals (Rugg-Gunn et al., 2011).

2.10.1.1 Urine collection

Some investigators (García-Hoyos et al., 2012, Akpata et al., 2014) have collected first morning urine as a representative of 24-hour urine but investigations (Zipkin et al., 1956, Warpeha and Marthaler, 1995) have shown that F concentration of a single spot urine sample is not a valid method for extrapolating to 24-hour urine collection. Akpata and co-workers (Akpata et al., 2014) in their study on F intake from fluids and urinary F excretion by young children in a non-fluoridated community in Kuwait mentioned the practical difficulties in collecting 24-hour urine samples from many children in their study design and therefore they estimated 24-hour urinary F excretion from the F/creatinine ratio of the early morning spot urine samples, a method described in a previous study (Zohouri et al., 2006a). Some studies have shown that the mean of three (Zipkin et al., 1956) or two (Bean et al., 1989) daily spot urine samples may be an accurate guide to the daily urinary F concentration but not daily urinary F excretion which provide important information about F retention. To obtain the most reliable estimate of daily urinary F excretion, total 24-hour urine should be collected because it allows the impact of the variation of urinary flow rate through different times of the day on the total 24-hour urine volume to be considered (Zohoori et al., 2013b). The 24 hour urine sample has been regarded as a reliable period of time for urine collection which is independent of dietary habits, timing of meals and periods of maximal F intake (WHO, 2014). However, the collection of 24 hour urine samples may not be a convenient method for monitoring F excretion of large groups of people within the community (Lennon et al., 1996). Due to difficulties in collecting 24-hour urine from children, the WHO (WHO, 2014) suggested a range of standards for the urinary F excretion of 3-14 year old children receiving low, optimum and high F (Table 2.6).

24-hour	Lower (mg F)	Upper (mg F)
Age 3-5 years		
Low F intake	0.17	0.29
Optimal F usage	0.36	0.48
Age 6-7 years		
Low F intake	0.19	0.31
Optimal F usage	0.48	0.60
Age 10-14 years		
Low F intake	0.22	0.34
Optimal F usage	0.60	0.82

Table 2-6: Standards indicating optimal exposure to F, recommended by WHO for 24-hour urinary F excretion (UFE) of different age groups. WHO (2014)

2.10.1.2 Validation of completeness of 24 hr. urine collection

The use of a biochemical measure of exposure to a nutrient is an alternative method of assessing dietary intake due to difficulties in obtaining accurate dietary information by dietary assessment methods. Biomarkers reflecting nutrient intakes such as F can be found in various biological media namely: urine, faeces, blood, sweat, hair and nails. The WHO stated that F biomarkers are of value primarily for identifying and monitoring deficient or excessive intakes of biologically available F (Selwitz, 1994). Monitoring F exposures through analysis of the various biological media is accompanied by varying degrees of accuracy (Rugg-Gunn et al., 2011). Urine is the most frequently employed biological medium in nutritional epidemiological studies because the majority of the biomarkers can be found in urine and its collection is relatively convenient and non-invasive. However, the complete and accurate collection of 24 hour urine should be verified to prevent incorrect results and conclusions. The completeness of the 24 hour urine collection can be verified by:

- Externally induced markers
- Internally produced markers

External markers such as lithium and para-amino benzoic acid (PABA) have been used orally a few days before the actual urine collection after which they are excreted in urine and measured (Sanchez-Castillo et al., 1987). Lithium was used as an external marker because of its low concentration in diet and its complete excretion in urine; a urine sample of > 95% recovery of lithium signified completeness of 24 hour urine collection (Bingham, 2003). However, with increased fluid intake the recovery of lithium decreased resulting in false conclusion that participant provided incomplete urine sample (Amdisen, 1977). PABA needs to be given to participants during breakfast, lunch and dinner. It is absorbed and excreted quantitatively within 24 hours and urine sample of < 85% of the ingested PABA is

regarded as incomplete (Bingham, 2003). However, there are concerns among parents regarding the side effects or safety of these substances for their children, some children may refuse to ingest these substances and their use is increasingly avoided.

Internal markers do not require substances to be ingested at certain times and are therefore more suitable for children. An internal marker such as creatinine is spontaneously produced from dephosphorylation of creatinine, a substance mainly found in muscle tissues. Creatinine has no biologic function and is steadily released from the muscle cells and excreted via the kidneys with minimum re-absorption (Litchford, 2008). Total 24 hour urinary creatinine has been suggested as a reliable measure of completeness of a 24 hour urine collection for healthy individuals with no muscle tissue loss due to dietary restrictions or injury and there are reference values for 24 hour urinary creatinine excretion in children based on weight, height and age (Table 2.7).

Author (Year)	**Suggested range**	**Parameters**
Tietz (1995)	8-22 mg/kg bw/day	Weight
WHO Marthaler (1999)	0.1-1.5 mg/ml	All ages
Remer et al. (2002)	Male • 4-5 years 17.08 mg/kg bw/day • 6-8 years 19.45 mg/kg bw/day • 9-13 years 20.58 mg/kg bw/day Female • 4-5 years 16.06 mg/kg bw/day • 6-8 years 18.09 mg/kg bw/day • 9-13 years 19.34 mg/kg bw/day	Weight, age & gender
Avner et al. (2009)	< 2 years 7.1-9.9 mg/kg bw/day 2-8 years 12.2-21.2 mg/kg bw/day 9-18 years 14.9-23.9 mg/kg bw/day	Weight, age

Table 2-7: Summary of suggested creatinine values based on different parameters.

Tietz (1995) suggested a wide range of 8-22 mg/kg bw/day creatinine excretion based on body weight while a range of 0.1-1.5 mg/ml that was not based on weight, height or age was proposed by WHO (WHO, 2014).

Another internal marker used to validate the completeness of 24 hour urine is urine flow rate. Flow rates of 5-160 ml/hour have been suggested by WHO for children younger than 6 years as normal while for children ≥ 6 years, 9-300 ml/hour has been suggested as normal (Table 2.8).

WHO	Lower limit	Upper limit
Urine flow		
2 – 4 years (ml/24 hours) 4 – 6 years (ml/24 hour)	140 200	- -
2 – 4 years (ml/hour) 4 - 6 years (ml/hour)	5 7	- -

Table 2-8: Suggested normal urine flow rates by WHO, (WHO, 2014)

Based on this reference, urine samples with volumes less than 140 ml/24hour should be discarded as being incomplete. Several studies (Marthaler et al., 1995, Ketley and Lennon, 2001, Maguire et al., 2007) that have used WHO recommendations to validate 24-hour urine collection reported that urine flow rates below or above the recommended limits were excluded due to incomplete collection. However, it should be noted that daily urine volume and consequently flow rate may be affected by the type and volume of liquid consumed per day.

In summary, urine can be collected through single spot and 24-hour samples but the latter provides a more reliable estimate of daily urinary F excretion. The collection of 24-hour urine samples places great burden on study participants and their parents or guardians and it is not convenient for monitoring large group of participants. The complete and accurate collection of 24-hour urine can be verified by measuring urinary excretion of substances that are ingested at certain times or produced from body metabolism and by measuring urinary flow rate. Although the 24 hour urine collection place a burden on participants, the validation of completeness using urinary flow rates is easier, practical, feasible and does not incur any significant additional cost.

2.10.2 Studies which have assessed urinary F excretion (UFE)

A number of urinary F excretion studies have been undertaken in both fluoridated and non-fluoridated areas and a summary of their findings is presented in Table 2.9. These studies have been undertaken during fluoridation programmes for water (Rugg-Gunn et al., 1993, Acevedo et al., 2007), salt (Warpeha and Marthaler, 1995, Marthaler et al., 2000, Acevedo et al., 2007) and milk (Ketley and Lennon, 2000, Villa et al., 2000, Ketley and Lennon, 2001). In addition, some of the studies investigated the influence of using fluoridated toothpaste on urinary F excretion (Ketley et al., 2004, Forte et al., 2008, Martins et al., 2011c). The urine collection method in the majority of studies was a 24-hour urine sampling method. This provides the most reliable estimate of UFE since it allows the impact of the variation of urinary flow rate through different times of the day on the total

24-hour urine volume to be measured. And more importantly, 24 hour urine collection allows the variation in F intake throughout the day to be assessed and is suitable for older children who are toilet trained. Other methods such as spot, F/Cr ratio are more suitable for younger children who wear nappies. In a small number of studies, usually those with larger numbers of participants, F excretion is estimated using spot urine or supervised collection of urine because of the logistical and practical difficulties associated with collection of 24 hour urine samples from larger groups of individuals. The majority of the UFE studies were undertaken among children <7 years of age. To date there has been no urinary F excretion study undertaken in Africa probably due to lack of research funds or skills. The majority of the UFE studies reported have been undertaken in water fluoridated areas with the aim of determining or monitoring the influence of F exposure through drinking water on urinary F excretion. Very few studies have reported UFE among children exposed to formula feeding, fluoridated milk or fluoridated salt. The reported daily urinary F excretion was 0.144 mg/day, 0.33 mg/day and 0.23-0.29 mg/day respectively for 0.19 to 0.89-year-old US formula-fed infants (Ekstrand et al., 1994b), 4-5 year-old UK children receiving F milk (Ketley and Lennon, 2000) and 3-4 year-old Swiss (Marthaler et al., 2000) children receiving F salt.

2.10.3 Urinary F excretion (UFE) in fluoridated areas

Table 2.9 shows that in fluoridated areas, UFE in children ranged from 0.229 mg/day in 3-5-year-old Chilean children living in 0.6 mg/l water F areas (Villa et al., 1999) to 3.100 mg/day in 8-9-year-old children in Mexico living in 2.77 mg/l water F areas (Grijalva-Haro et al., 2001) due to differences in age group studied and F concentration in water. Some studies (Grijalva-Haro et al., 2001, Maguire et al., 2007, Acevedo et al., 2007, Zohoori et al., 2013a) showed the influence of F concentration in water on UFE and since water is a primary source of fluid intake in the diet, urinary F excretion increases as the F concentration in water increases (Grijalva-Haro et al., 2001, Ketley et al., 2004, Maguire et al., 2007, Zohoori et al., 2013b). For example, the UFE of 6-7 year-old children exposed to 0.08 mg/l, 0.47 mg/l and 0.82 mg/l water F in UK was 0.203 mg/day, 0.239 mg/day and 0.323 mg/day respectively (Maguire et al., 2007). In addition, in a salt fluoridation programme among 3, 4 and 5 year-old Venezuelan children, the UFE of those who were exposed to higher water F was higher than those exposed to lower water F (Acevedo et al., 2007). The influence of F supplements on UFE was investigated in a study (Villa et al., 1999) among 3-5 year-old Chilean children exposed to fluoridated water (0.57-0.62 mgF/l).

In this study, when the Chilean children had F added to juices as an additional source of F intake, the UFE increased from 0.229 mgF/day to 0.526 mgF/day thereby showing the additive role of a F supplement and F concentration in water on UFE. Similarly, the influence of fluoridated toothpaste as additional source of F intake on UFE was observed among 2-7 year-old Brazilian children exposed to same water F concentration, those who used fluoridated toothpaste had higher UFE than their counterparts who used non-fluoridated toothpaste. In terms of the effects of nutritional status on F metabolism, urinary F excretion was assessed among 60 preschool Mexican children with and without malnutrition and the mean urinary F excretion was 367 μg/24 hour and 355 μg/24-hour respectively (Juárez-López et al., 2008). The slight differences in UFE seen in this study was not statistically significant, and the effects of malnutrition on F metabolism remain unclear (Buzalaf 2011).

The overall influence of F intake on UFE in fluoridated areas was investigated in a study (Martins et al., 2011c) on the effect of discontinuation of F intake from sub-optimally fluoridated water and toothpaste containing 1,100 ppm F on urinary excretion in 11 two – four years Brazilian children. In this study, the mean urinary F excretion was 0.25 mg/day during baseline and dropped to 0.14 mg/day during interruption, rising to 0.21 mg/day during re-exposure. The concentration of F in the human body may decrease and increase again when intake is interrupted and re-started since there is no homeostatic mechanism for maintaining circulating F. This shows that UFE is a reliable biomarker of F exposure and can be used to monitor F exposure in a population.

2.10.4 Fluoride excretion from low or non-fluoridated areas

Table 2.9 also shows the urinary F excretion in low or non-fluoridated areas by country, age and sources of F exposure. In low F or non-fluoridated areas, UFE in children ranged from 0.136 mg/day among 4-year-old Venezuelan children (water F = 0.12 mg/l and salt fluoridation = 60 mg/kg) (Acevedo et al., 2007) to 0.339 mg/day for 4-year-old Iranian children (water F=0.3 mg/l) (Zohouri and Rugg-Gunn, 2000b) due to differences F metabolism and altitude of residence of participants. Urinary F excretion is determined by rate of F uptake and removal from bone and the efficiency with which the kidneys excrete F (Buzalaf and Whitford, 2011). Alterations in acid-base balance caused by hypobaric hypoxia during residence at high altitude were cited as the cause of decreased urinary pH and reducing urinary F excretion and therefore greater F retention (Whitford, 1997). Despite age, racial, dietary and F exposure differences the mean urinary F excretion of 6-7-

year old UK children living in areas where the water F concentration was 0.08 mg/l was 0.203 mg/day (Maguire et al., 2007) was similar to those reported for 4 and 5-year-old Venezuelan children living in area where the water F concentration was 0.34 mg/l and the salt fluoridation was 60 mg/l (Acevedo et al., 2007). These similarities were most likely due to the high contribution of F intake from toothpaste ingestion in the UK children. In 5 European countries with water F concentrations of < 0.15 mg/l, the urinary F excretion was between 0.160 to 0.33 mg/day among 3-year-olds (Ketley et al., 2004); these differences being primarily due to differences in geographical locations, dietary and tooth cleaning practices.

In summary, UFE studies in fluoridated and non-fluoridated areas have reported a wide variation in urinary F excretion among various groups of children in various countries, but similar excretions when contributory factors in terms of F exposure, F metabolism and physiology are similar. Very few studies have reported urinary F excretion among 8-year-old children and there is dearth of F excretion studies in Africa. Information on urinary F excretion, fractional urinary F excretion and total daily F intake is necessary to estimate F retention, and F body balance, all of which impact on F body burden and the consequential risk of dental fluorosis and DDE.

2.11 Fractional urinary F excretion (FUFE)

The use of fractional urinary F excretion (FUFE) as the proportion of F ingested (TDFI) which is excreted aids the understanding of the relationship between F intake and excretion. This information is necessary both in the field of F metabolism and in epidemiological surveys which aim to estimate TDFI from urinary measurements. More assessment of the suitability and validity of UFE as a tool for monitoring fluoridation schemes as well as for predicting total F intake has been suggested (Zohoori et al., 2013b). Table 2.9 summaries UFE studies that have reported FUFE by country, age and F exposure. Not many studies have reported FUFE and the reason for this is that to determine FUFE, both F intake and F excretion data are needed for the same group of individuals and fewer studies are designed to collect both types of outcome data. In water fluoridated areas, the FUFE ranged from 30% to 80% reported for 6-7 year-old children in England living in water F areas of 1.01 mg/l (Zohoori et al., 2013b) and 4 year-old Iranian children who drank 0.33 mg/l water F (Zohouri and Rugg-Gunn, 2000b) respectively. These differences in FUFE may be due to differences in levels of F intake due to differences in the geographical locations, temperature, F concentrations found in the water supplies, other dietary practices, but in

addition, the age of the children studied and whether they were undergoing periods of active bone growth is also an important predictor of FUFE with greater F retention seen in children undergoing periods of active bone growth. Two studies (Maguire et al., 2007, Zohoori et al., 2013b) showed that F concentration in water was inversely related to FUFE, as the F concentration of water which children are exposed to increases the proportion of F intake which is excreted through urine decreases. This translates to higher F retention with increased F intake. In non-fluoridated areas of England, the FUFE was 40% reported for 6-7 year-old children living in water F areas of 0.30 mg/l (Zohoori et al., 2013b). A study that investigated the relationship between UFE and TDFI in relatively large number of children and adults reported that on average 45% and 74% of ingested F was excreted in urine in children and adults respectively thereby confirming lower F retention by increasing age.

FUFE was 30%, 78% and 51.5% reported for 4-5 year-old UK children who drank fluoridated milk (Ketley and Lennon, 2000), 0.19-0.89 year-old American infants who were formula-fed and 3-6 year-old German children whose food were cooked with fluoridated salt (Haftenberger et al., 2001). These differences in FUFE were also due to differences in dietary practices, geographical locations, but also, most importantly, the age range of study participants.

2.12 Fluoride retention (F balance) and its assessment

The difference between F intake and F excretion represents F retention and is also known as F balance. Practically, F retention is estimated by subtracting total F excretion through urine and faeces from total daily F intake. When the total F intake is more than the total F excretion, the F balance is positive therefore F is retained in the body. If the amount of F retained is higher than the amount of F required for normal tooth and bone development, then an individual is at risk of excessive deposition of F in bone as well as in any teeth developing at that time and an increased risk of dental fluorosis may be a consequence of this. Conversely, when the total F excretion is more than the F intake, body F balance is negative and can represent loss of F from the skeleton. The F balance of an individual or group of individuals can be determined when both total F intake and urinary F excretion are measured in same subject, however, few studies have measured both parameters, especially in children.

Author (year)	Country	Age (years)	N	Source of F	Urine collection	UFE		FUFE (%)	FFR (%)
						mg/day	mg/kg bw		
Europe									
Ketley and Lennon (2000)	UK	4-5	8	Milk (0.5 mg)	24-h	0.33	0.017	30	NR
Marthaler et al. (2000)	Switzerland	3-4	25	Fluoridated salt (250mg)	4 spot urines	0.23-0.29	NR	NR	NR
Haftenberger et al. (2001)	Germany	3-6	11	Fluoridated salt	24-h	0.476	0.026	51.5	NR
Ketley et al. (2002)	Ireland UK	1.8-5.2	19 22 16	Water Ireland (0.8-1mg/l) Water UK (<0.1 mg/l) Milk UK (0.5mg)	24-h	0.36 0.21 0.30	NR	NR	NR
Ketley et al. (2004)	Ireland England Finland Iceland Netherland Portugal	3	19 18 18 4 6 21	DW (0.8-1.0 mg/l) DW (<0.15 mg/l) DW (<0.15 mg/l) DW (<0.15 mg/l) DW (<0.15 mg/l) DW (<0.15 mg/l)	24-h	0.370 0.200 0.160 0.170 0.210 0.330	0.022 0.014 0.011 0.011 0.014 0.022	NR	NR
Zohouri et al. (2006b)	UK	1-3	7	DW (0.8 mg/l)	Spot urine	0.33		48	NR
Maguire et al. (2007)	UK	6-7	18 8 5	DW (0.08 mg/l) DW (0.47 mg/l) DW (0.82 mg/l)	24-h	0.203 0.239 0.323	0.008 0.011 0.014	44 40 32	NR
Zohoori et al. (2013b)	England	6-7	21 12	Low water areas (0.30 mg/l) Natural water areas (1.01 mg/l)	24-h		0.012 0.017	40 30	NR
North America									
Ekstrand et al. (1994b)	USA	0.19-0.89	4	Formula fed	NR	0.144		78	12.5
Baez et al. (2000)	USA	4-6	31	DW – School (1.0-1.3 mg/l) DW – Home (0.1-3.2 mg/l)	Supervised collections	0.750	0.042	NR	NR
South America									
Villa et al. (1999)	Chile	3-5	42 46	DW(0.57-0.62 mg/l) DW (0.57-0.62+F supplement (1 mg in 50 ml orange juice)	24-h	0.229 0.526	0.015 0.028	NR	NR

Author (year)	Country	Age (years)	N	Source of F	Urine collection	UFE		FUFE (%)	FFR (%)
						mg/day	mg/kg bw		
Villa et al. (2000)	Chile	3-5	20	DW (0.5-0.6 mg/l)	24-h	0.358	0.022	35.5	54
Grijalva-Haro et al. (2001)	Mexico	8-9	11 10 11	DW (0.54 mg/l) DW (0.78 mg/l) DW (2.77 mg/l)	24-h	0.930 1.040 3.100	0.034 0.038 0.115	61 45 57	NR
Franco et al. (2005b)	Colombia	4-5	96	Table salt (180-220 mg/kg)		0.414		33	NR
Acevedo et al. (2007)	Venezuela	3 4 5	8 10 11 11 12 8	DW (0.12 mg/l) + Salt* DW (0.34 mg/l) + Salt* DW (0.12 mg/l) + Salt* DW (0.34 mg/l) + Salt* DW (0.12 mg/l) + Salt* DW (0.34 mg/l) + Salt*	24-h	0.188 0.273 0.136 0.203 0.207 0.287	0.013 0.019 0.009 0.013 0.011 0.016	NR	NR
Forte et al. (2008)	Brazil	2-7	10 17 15	DW (0.5-1.0 ppm) + FT DW (0.5-1.0 ppm) + NFT DW (1.1-1.5 ppm) + FT DW (1.1-1.5 ppm) + NFT DW (> 1.5 ppm) + FT DW (> 1.5 ppm) + NFT	24-h	0.453 0.435 0.451 0.430 0.592 0.623	NR	NR	NR
Zohoori et al. (2013a)	Brazil	<4	14 15	DW(0.6-0.8 µg/L) DW(<0.3 µg/L)	24-h & faeces	NR	0.026-0.039 0.005-0.008	NR	0.33 0.84
Middle East									
Zohouri and Rugg-Gunn (2000b)	Iran	4	78	DW (0.33mg/l)	24-h	0.339	0.024	80	11
Akpata et al. (2014)	Kuwait	1-9	404 4	Tap water (0.04 ppm)	Spot urine	0.13-0.22	NR	NR	NR
Multi-country									
Rugg-Gunn et al. (1993)	UK Sri Lanka	4	44 53	DW (0.8-1.1 mg F/L)	24-h	0.42 0.55	NR	NR	NR

Table 2-9: Studies of urinary F excretion (UFE) conducted in both fluoridated and non-fluoridated areas.

*Notes: n – Number; [1]UFE – Urinary F Excretion, [2]FUFE – Fractional Urinary Fluoride Excretion; [3]FFR – Fractional Fluoride Retention; DW – Drinking water, FT – Fluoridated toothpaste (1510 ppm), NFT – Non-fluoridated toothpaste; * (60-90 mg/kg); NR – Not reported.*

F retention depends upon a number of factors, including some dietary factors that can increase or reduce the absorption and excretion of F (Buzalaf and Whitford, 2011). In the absence of high concentrations of certain cations such as Ca, Fe and Al, almost 90% of F ingested with food is absorbed from the gastro-intestinal tract and passed rapidly into the blood (Zohoori et al., 2013b). The remaining 10% is excreted with the faeces though urinary F excretion is the most important metabolic pathway for F elimination from the body (Ekstrand et al., 1984, Ekstrand et al., 1994b). Calcium, iron and magnesium can form insoluble complexes with F which can significantly reduce F absorption while protein and fat reduces gastric emptying which then increases F absorption (Cerklewski, 1997). It is generally accepted that F retention occurs almost entirely in hard tissues (hard tissues such as teeth and bones have high affinity for F ions) and that F retention in soft tissues is almost negligible (Whitford, 1990, Whitford, 1996a). The proportion of ingested F that is retained has been thought to be around 50% (WHO, 1994b) but it is clear that it varies with age and active growth periods and in response to metabolic and dietary factors (WHO, 1994b).

In a study (Brunetti and Newbrun, 1983) in children aged 3 to 4 years residing in a fluoridated community in California, USA, the net F retention of these children was + 0.05 mg/day because the F intake was 0.33 mg/day and greater than F excretion in urine and faeces (0.28 mg/day). In a report on appropriate use of F for human health, Murray (Murray, 1986) suggested that 70% of ingested F was retained by preschool children, however, Zohouri and Rugg-Gunn in a F intake and excretion (FIE) study of 78 children in Iran found that only 20% was retained by 4 year olds (Zohouri and Rugg-Gunn, 2000b). An even lower proportion of 12.5% retention was reported in a F balance study among 11 breast-fed American infants (Ekstrand et al., 1994b), while a study on 3-5-year-old Chilean children showed that 55% of ingested F was retained in the body (Villa et al., 2000). In the UK, a study of 6-7-year-olds showed that F retention was 58%, 50% and 46% for children living in optimally, sub-optimally and non-fluoridated areas respectively (Maguire et al., 2007) with a strong positive correlation between total daily F intake and F retention. Influence of diet on urinary F excretion might be the reason for the observed differences because meat based diets promote more acidic urine resulting in increased urinary F excretion and reduced F retention while vegetarian diet render urinary pH alkaline leading to reduced urinary F excretion and increased F retention.

Several studies on F balance have been undertaken in various parts of the world among various populations in fluoridated and low F areas. However, there is dearth of these studies

in Asia, Africa and Australasia and few studies have reported these parameters among 8 year olds.

2.13 Determination of F concentration of biological and non-biological samples

The F selective electrode has become a widely used method for determining F in a variety of samples because of its excellent performance, speed and general convenience. However, it does not respond to covalently bound F either as inorganic salts such as monofluorophosphate or as organic fluorine compounds. This bounded F must be cleaved to release F ions prior to measurement with the electrode. This cleavage involves use of acids and enzymatic hydrolysis using phosphatase enzyme. Therefore, before measurement of F, several techniques such as open ashing (Singer et al., 1980), digestion with acid and use of sodium biphenyl (Venkateswarlu, 1990) have been reported for pre-treatment of samples in order to release F ions from other matrices in the sample and to remove organic matter which may interfere with F analysis. Furthermore, F ions that are released need to be separated and concentrated by several techniques such as distillation (Willard and Winter, 1933), diffusion (Venkateswarlu, 1992), reverse extraction (Venkateswarlu, 1974), anion exchange (Kelso et al., 1964) and adsorption (Venkateswarlu and Sita, 1971). After fluorides have been separated and concentrated in soluble inorganic form, they are measured quantitatively by the following methods namely: colometric method (Trimetric and spectrometric) (Williams, 1946), gas chromatography (Bock and Semmler, 1967), aluminium monofluoride molecular absorption spectrometry (AIF MAS) (Venkateswarlu, 1992), fluorometry (Powell and Saylor, 1953) and F selective electrode (Powell and Saylor, 1953). In view of its performance and reliability it was the F-ISE which was used throughout the current study and further appraisal and consideration of its use is given in Chapter 4.

2.14 Fluoride intake and dental caries

The widespread epidemiological studies of (Dean, 1940), around the middle of last century found a strong inverse relationship between F exposure and the prevalence and progression of dental caries thereby establishing the role of F in the prevention of dental caries. Caries experience has decreased significantly due to F use from various sources and artificial adjustment of F exposure in water in developed countries over recent decades (Buzalaf and Levy, 2011). F in drinking water exerts a cariostatic effect and several studies have verified the cariostatic effect of a higher F concentration in drinking water (Ibrahim et al., 1997, Angelillo et al., 1999, Tsutsui et al., 2000) whereas other studies have found either no such

effect (Grobler et al., 2001, Wondwossen et al., 2004, Birkeland et al., 2005) or only a negligible effect (Meyer-Lueckel et al., 2006). In a study (Szpunar and Burt, 1988) on dental caries, fluorosis and F exposure among 6 to 12 year old Michigan school children, the mean DMFT was 1.99, 1.54, 0.87 and 0.74 in a F-deficient, 0.8 ppm, 1.0 ppm and 1.2 ppm community respectively. The corresponding value for prevalence of fluorosis was 12%, 32%, 49% and 51%. Similarly, in a study of dental caries and enamel fluorosis among the fluoridated and non-fluoridated populations in the Republic of Ireland, caries experience was lower while dental fluorosis prevalence was higher among children using fluoridated domestic water supplies than their counterparts who used non-fluoridated water supplies. In contrast, a higher prevalence of both caries and fluorosis have been reported for high F areas in Sudan (2.56 ppmF) (Ibrahim et al., 1997, Birkeland et al., 2005) and Ethiopia (Wondwossen et al., 2004) although this may have been due to the presence of more severe dental fluorosis which encourages cariogenic activity or an inability to differentiate between caries and fluorosis.

A cross-sectional study to determine the association between social deprivation and the prevalence of caries and enamel fluorosis among 1683 11-to-13 year old children in areas served by either fluoridated or non-fluoridated drinking water in the UK showed that water fluoridation reduced dentinal caries as well the social class gradient of caries experience (McGrady et al., 2012a). The anti-caries effects of standard F toothpastes are well established but their use among pre-schoolers (2-to-5-years) has given rise to concerns regarding the development of dental fluorosis and increasing support for lower F (around 500ppmF) toothpastes has been recommended in some countries (Santos et al., 2013). However, a recent systematic review of effects of low and standard F toothpastes on caries and fluorosis showed no evidence to support the use of lower F toothpastes by pre-schoolers regarding caries and fluorosis prevention (Santos et al., 2013).

There is still some uncertainty around the inverse relationship between F exposure and the occurrence of dental caries, therefore, further studies are needed to further explore this relationship.

2.15 Genetic interactions in F metabolism

There is a general consensus that the severity of dental fluorosis increases with increasing F exposure (Yadav et al., 2009, Everett et al., 2009) however, individual variation in dental fluorosis severity can occur when F exposure is relatively constant in a community (Mabelya

et al., 1994). Genetic factors may cause an increased susceptibility to dental fluorosis or produce conditions such as amelogenesis imperfecta that mimic dental fluorosis (Vieira et al., 2005). Some studies (Russell, 1962, Butler et al., 1985, Beltrán-Aguilar et al., 2005, Martinez-Mier and Soto-Rojas, 2010) have reported racial differences in the susceptibility to dental fluorosis; it is higher among African-American children in the USA. In the epidemiological survey undertaken in 1980–81 among 2592 7-to-19 year old lifetime residents of 16 towns with F concentration ranging from 0.2-3.3 ppm in Texas, Butler and colleagues (Butler et al., 1985) discovered that children of African-American descent had 2.3 times the risk of developing dental fluorosis when compared to Hispanic and non-Hispanic white children.

In order to evaluate the role of certain genetic factors in the occurrence of dental fluorosis, Liu et al (Liu et al., 2006) and Huang et al (Huang et al., 2008) studied dental fluorosis among Chinese children who were lifetime residents in areas with the same water F concentration and reported that some children had fluorosis while others did not. In the Liu et al study (Liu et al., 2006) among thirty 10–12 year olds, in populations from two residential areas of China with different concentrations of F in their drinking water (1.1–2.0 mg F/L in one village and 0.76 mg F/L in another), 1057 genes were differentially expressed in the children with and without fluorosis from the high F town. Some genes were robustly up-regulated while some were robustly down-regulated. These genes included transcription factors, signal transduction and cancer genes, structure and transport proteins and genes related to immunity and apoptosis (Liu et al., 2006).

In their case-control study among 240 eight and twelve year old children with and without dental fluorosis Huang and colleagues reported that about 50% of the children with high F exposure suffered from dental fluorosis and concluded that a PvuII polymorphism (rs414408) within the COLIA2 gene may be associated with the increased risk of dental fluorosis in high-F-exposed populations (Huang et al., 2008). A study of the relationship between a further polymorphism (Rsa I in Estrogen receptor (ER – rs4266)) and children's dental fluorosis reported no relationship between the ER Rsa genotype between cases and non-dental fluorosis in endemic fluorosis areas, however, children carrying the R allele had a higher risk compared with children carrying the r allele (Ba et al., 2009b). Ba et al. (2011) also provide evidence of an association between this polymorphisms in the ER gene and dental fluorosis in high-F-exposed populations. An earlier study had shown that oestrogen played an important role in stimulating osteoblast activity and promoting the deposition of

calcium and phosphate in bone (Scheven et al. 1992). Similarly, genes involved in oestrogen metabolism and activity were earlier shown to be strong candidates that explained at least in part the genetic influence in bone mineral density (Riancho et al. 2006). A case-control study (Wen et al., 2012) on the relationship of parathyroid hormone (PTH) gene Bst BI a polymorphism, calciotropic hormone levels and dental fluorosis of children in China showed no correlation between dental fluorosis and carriers of the Bst BI but serum osteocalcin may be a more sensitive biomarker for detecting early stages of dental fluorosis and further studies are needed. In contrast, a study on the association between osteocalcin gene polymorphism and dental fluorosis among children exposed to F in China showed that osteoclacin HindIII polymorphisms may not be a useful genetic marker for differential risk of dental fluorosis among children in China (Ba et al., 2009a).

Many studies in animals (Everett et al., 2002, Vieira et al., 2005, Carvalho et al., 2014) have also shown the possibility of a genetic component in susceptibility to dental fluorosis. Everett et al. (2002) assessed the occurrence of dental fluorosis in 12 three-week old inbred species of male weaning mice by grouping them into three treatment groups: one group was given distilled water while the other 2 groups had distilled water with 25 ppm F and 50 ppmF. On a weekly basis, each mouse had a complete oral examination of the entire upper and lower incisor tooth surfaces for fluorosis using the TF scoring system. With the use of Quantitative Light induced Fluorescence (QLF) the presence of fluorosis in extracted mandibular central incisor was confirmed. Further examination of processed mineralized tissues removed from experimental mice at day 60 showed that at 50 ppm F all strains developed various levels of dental fluorosis while at 25ppmF only some strains showed fluorosis. Overall, a particular strain of mice (A/J) showed early susceptibility to dental fluorosis at both 25 ppm F and 50 ppm F while another strain (129P3/J) showed minimal fluorosis even at 50 ppmF. In a study on renal proteome in mice with different susceptibilities to fluorosis, Carvalho et al. (2014) reported that proteomic analysis was able to identify potential proteins which could contribute to understanding the molecular mechanisms underlying genetic susceptibility to dental fluorosis. Similarly, Zhang et al (Zhang et al., 2014b) in an animal study of the effects of F on ameloblasts concluded that production of cellular diactlglycerol (DAG) was significantly increased in fluorosed ameloblasts suggesting that the increased phosphorylation of special AT-rich sequence-binding protein-1 (SATB1) gene may be related to an effect of F in enhancing Gαq activity of secretory ameloblasts.

Vieira et al. (2005) carried out a study to ascertain the influence of genetic and environmental factors in the occurrence of dental fluorosis in three species of mice (A/J, 129P3/J and SWR/J) known to have different degrees of susceptibility to dental fluorosis. Neutron activation analysis was used to measure F concentration of teeth of different groups of weaning mice exposed to 6 weeks of drinking water containing F levels of 0, 25, 50 and 100 ppm. Dental fluorosis was assessed by QLF and tooth quality was determined by enamel and dentine micro-hardness and dentin mineralization testing. Generally, dental fluorosis was directly proportional to the F level in drinking water and the prevalence of fluorosis was higher among A/J mice compared to SWR/J mice despite similar enamel hardness. Furthermore, severity of dental fluorosis was associated with F concentration in teeth, but only 34% of the variance was explained by the tooth F (Vieira *et al.*, 2005). This work showed that genetic factors can be associated with severity of dental fluorosis. A recent atomic force microscopy study on enamel crystals of mice susceptible or resistant to dental fluorosis showed that enamel crystals of the 129P3/J strain are narrower which is indicative of slower crystal growth and could interfere in the occurrence of dental fluorosis (Buzalaf et al., 2014).

Paradoxically, since dental fluorosis is related to an effort to prevent dental caries by adding F to drinking water, using fluoridated toothpaste etc., the identification of relatively more fluorosis susceptible populations may be a means of mitigating some of the burden (Ba et al., 2011). More research is needed to provide information about the potential genetic biomarker and associated increased risk of developing dental fluorosis in populations exposed to high F. An analysis and evaluation of the Huang et al study (Huang et al., 2008) of COLIA2 polymorphism and dental fluorosis stated that their results should stimulate additional investigations of potential genetic effects including considerations of possible interactions with F intake levels (Dawson, 2010). Better understanding of genetic factors contributing to dental fluorosis may illuminate both the developmental and pathogenic processes. This information could be used to identify -high-risk populations that are genetically susceptible to dental fluorosis which would help to guide decisions concerning the optimal use of F at community as well as at the individual level.

2.16 Overall Summary

Environmental and genetic factors can cause disturbances to tooth development resulting in defective enamel but further research among different populations is needed to understand the

role of genetic and environmental insults in the occurrence of development enamel defects. Certain classifications and terms have been used to describe developmental enamel defects and develop indices for measuring these defects directly or indirectly in epidemiological surveys among various populations. The findings from various epidemiological surveys of developmental defects of enamel and dental fluorosis shows that the prevalence and severity of these defects varies between groups according to timing and intensity of exposure to aetiological and risk factors. In addition, there was scarcity of studies on the pattern and distribution of these defects in primary teeth, a dearth of literature on the occurrence of developmental defects in sub-Saharan Africa and some uncertainty over the relationship between developmental defects of enamel, dental fluorosis and dental caries that requires further study.

There is wide variation in systemic F exposure from both dietary and non-dietary sources of F as well as the amount of drinks and foods consumed by children in different countries. There was a scarcity of data on F content of drinks and foods in Africa which translated into scarcity of information on systemic F intake and the contribution of drink and food to total daily F intake in sub-Saharan African populations. Information on F intake among 8 year olds was also limited. The wide variability in urinary F excretion, F retention and fractional urinary F excretion reported among various population groups in different parts of the world is clear but there was very limited data for >6 year olds and from Asia, Africa and Australasia. Studies have reported individual variation and racial differences in the susceptibility to dental fluorosis with a small number of reports on the association between polymorphism within certain genes such as COL1A2 gene and dental fluorosis. In view of these findings in the literature review the need for an observational study on defects of enamel and associated factors in a sub-Saharan African population is clear.

Chapter 3 Aim and Objectives of the study.

This study investigated the influence of F exposure through water, toothpaste and diet, dental factors as well as other environmental factors and genetic background of individuals on the occurrence of developmental defects of enamel and dental fluorosis among 4 and 8 year olds living in Nigeria.

3.1 Aim

To determine the influence of fluoride exposure (intake and excretion), nutritional deficiencies, dental factors as well as other environmental factors and genetics on developmental defects of enamel and dental fluorosis among 4 and 8 year olds in Nigeria with a view to improving the public health measures concerning exposure to fluoride at a community and individual level.

3.2 Objectives

1. To determine the prevalence and severity of developmental defects of enamel and dental fluorosis among the study participants.
2. To determine the relative contributions of different components of the diet and toothpaste to fluoride exposure
3. To determine the relationship between fluoride exposure and urinary fluoride excretion.
4. To examine the relationship between the prevalence and severity of dental fluorosis and burden of fluoride exposure.
5. To investigate the relationship between nutritional deficiency and fluoride excretion and retention.
6. To determine the relationship between single nucleotide polymorphism in the COL1A2 gene and the occurrence of dental fluorosis.

To address the main aim and objectives of this study, the study was carried out in 3 Phases:

Phase 1- Dental Health and Nutrition: to determine the prevalence and severity of developmental defects of enamel, dental fluorosis and dental caries and factors associated with the defects among 4 and 8 year olds in Nigeria.

Phase 2 - Estimating fluoride exposure: to estimate F exposure among a subgroup of the Phase 1 study participants by measuring F intake from diet and toothpaste and determined urinary F excretion.

Phase 3 - Single Nucleotide Polymorphism: to determine the relationship between single nucleotide polymorphism in the COL1A2 gene and the occurrence of dental fluorosis.

Chapter 4 The Pilot study and an overview of materials and methods for the main study.

4.1 Introduction

This chapter provides a description of the pilot study and overall materials and methods used to carry out this study, necessary to inform the reader and to ensure that the study could be replicated by other researchers, if necessary. The study used a cross-sectional observational survey design to determine the prevalence of developmental defects of enamel and dental fluorosis and factors associated with the occurrence of these defects among 4 and 8 year olds. Data collection and analyses were carried out to address the research questions described in Chapter 3: (a) presence of developmental defects of enamel and dental fluorosis; (b) dietary intake recording and 24h urine sample collection with measurement of fluoride (F) concentration in food, drink toothpaste and urine samples and (c) buccal swab collection, DNA extraction and gene sequencing to determine single nucleotide polymorphism. The pilot study and overview of overall methodology, a description of the study location, estimation of sample size, recruitment of study participants and ethical issues addressed is described.

4.2 Materials and methods

4.2.1 *Main Study design*

This study was carried out in three Phases, namely:

- Phase 1 – A cross-sectional study among parents/legal guardians and their 4 and 8 year-old children/wards about the presence of developmental defects of enamel, dental fluorosis and dental caries and factors associated with their occurrence;
- Phase 2 – A F intake and excretion study in a subsample of children estimating total F intake from diet and toothpaste ingestion as well as estimating urinary F excretion by collecting a 24 hour urine sample and;
- Phase 3 – Gene sequencing from DNA extracted from buccal mucosa cells of a subsample of children to provide information on single nucleotide polymorphism.

4.2.2 *Study location– Pilot study to determine main study location*

Oyo State was selected because it is where the University of Ibadan is located and a previous report (Ibiyemi and Taiwo, 2011) where Dental Fluorosis was demonstrated in some areas of the state. In addition, anecdotal observations (personal communication) have

shown the frequent occurrence of developmental defects of enamel and dental fluorosis among children in Ibadan and Ibarapa. In order to identify rural and urban populations residing in low and high fluoride areas, it was necessary to conduct a pilot study of the F concentration of water supplies in a number of areas within Oyo State, Nigeria. The study location, as shown in Figure 1, was chosen by randomly selecting one senatorial district out of the three senatorial districts in Oyo State, Oyo South Senatorial District made up of 15 Local Government Areas (LGAs) comprising rural and urban populations. A list of LGAs that contained urban and rural populations was drawn up after which one urban (Ibadan North) and one rural (Ibarapa Central) LGA were randomly selected by balloting. Ibadan North LGA is one of the 5 LGAs in Ibadan municipality, it has an area of 27km^2, a population of 308,119 (National Bureau of Statistics, 2010), and 12 wards populated by people of diverse socio-economic characteristics, the majority of adults being traders and artisans (Osoba, 2013). Ibarapa Central LGA comprised 10 wards occupying an area of 440km^2 with a population of 103,243 and lies approximately 80 kilometres south of Ibadan (Olawale and Owoaje, 2007). The majority of the residents in Ibarapa were the native local speakers (Yoruba) and their main occupations were farming and trading (Olawale and Owoaje, 2007).

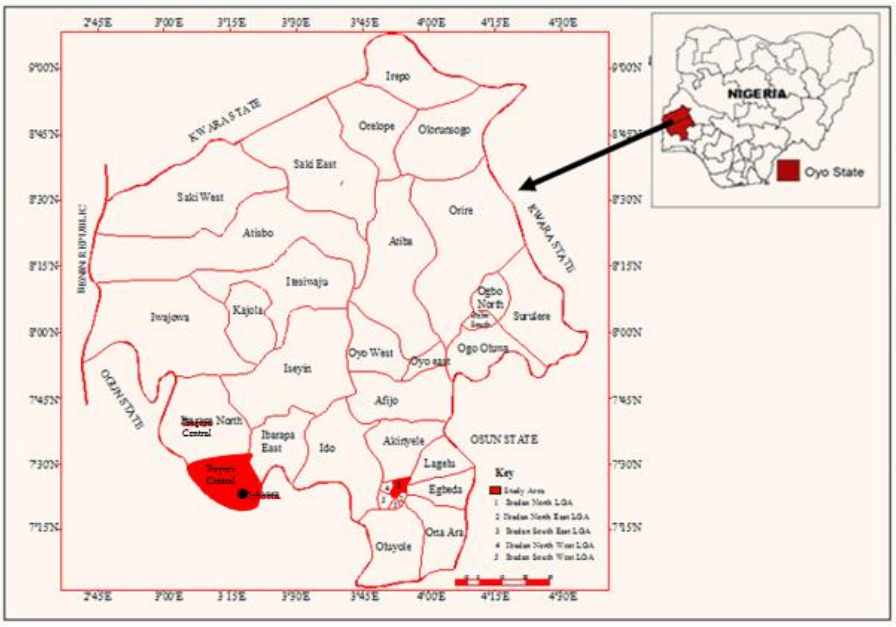

Figure 4-1: Map showing Ibadan North and Ibarapa Central Local Government Areas.

In each of the wards in Ibadan North and Ibarapa Central LGAs, determination of the actual study locations was carried out through a 2 stage pilot study:

4.2.2.1 Stage 1 of Pilot study

For each ward in the LGA, a 30 ml of water sample was obtained from 2 identifiable common sources of drinking water (wells or boreholes) after rinsing the collection bottle with the source water 3 times. In total, 44 water samples were collected and analysed for F concentration using a F-Ion Selective Electrode (F-ISE) in the oral pathology laboratory at the University of Ibadan, Nigeria by a direct method (Martínez-Mier et al., 2011) described in F analysis training manual (Omid et al., 2011). Prior to the direct F measurement in Nigeria, the researcher was trained on how to measure F concentration using a F-ISE by direct method at the F research laboratory, Newcastle University. After the water F analysis, mean values were calculated for each ward in the LGAs as shown in Appendix C. The results showed a wide variation in F concentration of waters in the 2 LGAs; 0.04 to 1.0mg/L in Ibadan North and 0.09 to 2.0 mg/L in Ibarapa Central LGA.

4.2.2.2 Stage 2 of Pilot study

The 4 wards with the highest (Wards 2, 6, 10 and 12) and lowest (Wards 3, 5, 7 and 8) mean water F concentration from Stage 1 were selected from Ibadan North LGA for Stage 2. Similarly, 4 wards (wards 1, 2, 3 and 8) with the highest and 4 wards (Wards 4, 5, 6 and 7) with the lowest mean water F concentration in Ibarapa Central LGA were selected for Stage 2. A total of 80 water samples (30 ml) for F analysis were obtained from a further 5 identifiable common sources of drinking water in each of these 16 selected wards. After F analysis, the mean values for F concentration of waters in each ward were calculated.

The results showed a wide variation in F concentration of waters in the 2 LGAs; 0.03 to 1.0mg/L in Ibadan North and 0.06 to 3.0 mg/L in Ibarapa Central LGA (Appendix D). Appendix D also shows the mean water F concentration in Ward 6 in Ibarapa Central LGA, a low water F concentration area, was 0.89 mg/L which was higher than mean values of Wards 2, 3 and 8; high water F concentration areas.

4.2.2.3 Results of stage 1 and 2 of the Pilot study

The summary data set for all water samples collected in Stages 1 and 2 for the 8 Wards in Ibadan North and Ibarapa Central LGAs is shown in Table 4.1. The results showed a wide variation in F concentration of waters in the 2 LGAs; 0.03 to 1.0 mg/L in Ibadan North and 0.06 to 3.0 mg/L in Ibarapa Central LGA.

4.2.3 *Planning the Main Study based on results of the Pilot Study*

4.2.3.1 Selection of location for main study

Galagan and Vermillion (1957) suggested a formula for calculating the appropriate F concentration in drinking waters for different climatic conditions depending on the ambient temperature. For tropical countries with mean maximum ambient temperature higher than 27^{o}C, a water F concentration of 0.6-0.7 mgF/L has often been recommended as appropriate based on the Galagan and Vermillion's formula. On this basis, in this study, 0.6mgF/L was set as the cut-off for optimal water F; areas with water F < 0.6 mg/L were selected as low water F areas and ≥ 0.6 mg/L as high water F areas for the main

4.2.3.2 Ibadan North LGA (Urban setting)

Based on Table 4.1, areas with water F concentration ranging between ≥ 0.6 mg/L and ≤ 0.09 mg/L in Ibadan North LGA were selected as high and low water fluoride areas respectively for the main study. A list of the number of public and private nurseries and primary schools located within 1 kilometre of the central part of these areas was obtained from the Local Government Secretariat (Table 4.2). All the public and private primary schools had nurseries.

4.2.3.3 Ibarapa Central LGA (Rural setting)

Similarly, in Ibarapa Central LGA, areas with water F concentration between 1.0 mg/L and 3.0 mg/L and between 0.06 mg/L and 0.1 mg/L were selected as high and low water F areas respectively (Table 4.1). A list of the public and private nurseries and primary schools located within 1 kilometre of the central part of these areas was obtained from the Local Government Secretariat (Table 4.3).

4.2.4 Selection of primary schools and nurseries for the main study

In both Ibadan North LGA (Urban setting) and Ibarapa Central LGA (Rural setting), study participants were selected from primary schools and nurseries from areas with highest and lowest water F concentration as described in Tables 4.2 and 4.3.

Male and female children aged 4 and 8 years residing in the selected rural and urban communities and living within walking distances of their nurseries and primary schools were invited to participate in the study. The 4 year olds were selected because they had fully erupted their primary teeth and these teeth were not expected to have exfoliated. The 8 year olds were selected because their permanent incisors and first molars would be fully erupted.

Local Government Area	Ward No.	Stage 1		Stage 2					Mean (SD) (mg/L)
		Area 1	Area 2	Area 3	Area 4	Area 5	Area 6	Area 7	
Ibadan North (Highest F conc. from Stage 1)	2	(W) 0.20	(B) 0.40	(W) 0.60	(B) 0.40	(B) 0.20	(W) 0.20	(W) 0.40	0.34 (0.15)
	6	(W) 0.30	(B) 0.50	(B) 0.30	(B) 0.80	(W) 0.30	(B) 1.00	(W) 0.20	0.40 (0.30)
	10	(B) 0.30	(W) 0.20	(B) 0.03	(W) 0.09	(W) 0.20	(B) 0.06	(W) 0.08	0.14 (0.10)
	12	(W) 0.50	(B) 1.0	(B) 0.04	(W) 0.03	(W) 0.20	(B) 0.15	(W) 0.30	0.32 (0.34)
Ibadan North (Lowest F conc. from Stage 1)	3	(B) 0.04	(B) 0.09	(B) 0.10	(W) 0.20	(W) 0.25	(B) 0.20	(W) 0.20	0.14 (0.08)
	5	(B) 0.09	(B) 0.07	(W) 0.30	(B) 0.20	(W) 0.19	(B) 0.20	(B) 0.10	0.16 (0.08)
	7	(B) 0.20	(B) 0.10	(W) 0.30	(W) 0.20	(W) 0.40	(B) 0.10	(W) 0.20	0.21 (0.11)
	8	(W) 0.20	(W) 0.10	(W) 0.30	(B) 0.20	(W) 0.20	(W) 0.10	(B) 0.20	0.19 (0.07)
Ibarapa Central (Highest F conc. in Stage 1)	1	(W) 0.30	(B) 2.00	(B) 2.00	(B) 0.30	(B) 3.00	(W) 2.00	(W) 0.30	1.41 (1.10)
	2	(W) 2.00	(B) 2.00	(W) 0.90	(W) 0.60	(W) 0.50	(B) 0.90	(W) 0.60	1.07 (0.65)
	3	(B) 0.30	(W) 1.00	(W) 0.30	(B) 0.30	(B) 0.30	(W) 0.40	(W) 3.00	0.80 (1.00)
	8	(B) 0.40	(B) 0.30	(W) 0.35	(B) 0.25	(W) 0.40	(B) 0.30	(W) 0.45	0.35 (0.07)
Ibarapa Central (Lowest F conc. in Stage 1)	4	(B) 0.20	(W) 0.20	(W) 0.09	(W) 0.09	(W) 0.10	(B) 0.07	(W) 0.10	0.12 (0.05)
	5	(B) 0.10	(W) 0.10	(B) 0.50	(W) 0.20	(B) 0.15	(W) 0.06	(B) 0.20	0.19 (0.15)
	6	(W) 0.40	(B) 0.20	(B) 0.80	(W) 0.60	(B) 0.75	(W) 2.00	(W) 0.30	0.72 (0.61)
	7	(W) 0.20	(B) 0.40	(W) 0.60	(B) 0.07	(W) 0.20	(B) 0.50	(B) 0.90	0.41(0.29)

Table 4-1: Mean (±SD) fluoride concentration (mg/L) of water collected from wells (W) and boreholes (B) in the highest and lowest mean water fluoride concentration wards in Ibadan North (Urban setting) and Ibarapa Central (Rural setting) LGAs (Stages 1 and 2).

LGA	Ward No.	Area	Type of water	F conc (mgF/L)	Public	Private
Ibadan North (Highest F conc. in water)	12	2	Borehole	1.00	3	2
	6	6	Borehole	1.00	2	2
	6	4	Borehole	0.80	2	1
	2	3	Well	0.60	2	2
Ibadan North (Lowest F conc. in water)	3	1	Borehole	0.04	3	3
	5	2	Borehole	0.07	2	1
	3	2	Borehole	0.09	3	2
	5	1	Borehole	0.09	2	3

Table 4-2: Number of public and private nurseries and primary schools and water F concentration (mg/L) in wells and boreholes in selected high and low water F areas in Ibadan North LGA (Urban setting).

LGA	Ward No.	Area No.	Type of water	F conc. (mgF/L)	Public	Private
Ibarapa Central (Highest F conc. in water)	1	5	Borehole	3.00	4	2
	3	7	Well	3.00	3	2
	1	3	Borehole	2.00	4	2
	1	6	Well	2.00	4	2
	2	1	Well	2.00	3	2
	2	2	Borehole	2.00	3	2
	1	2	Borehole	2.00	4	2
	3	2	Well	1.00	3	2
Ibarapa Central (Lowest F conc.)	5	6	Well	0.06	3	2
	4	6	Borehole	0.07	2	2
	4	3	Well	0.09	2	2
	4	4	Well	0.09	2	2
	5	1	Borehole	0.10	3	2
	5	2	Well	0.10	3	2
	4	5	Well	0.10	2	2
	4	7	Well	0.10	2	2

Table 4-3: Number of public and private nurseries and primary schools and water F concentration (mg/L) in wells and boreholes in selected high and low water F areas in Ibarapa Central LGA (Rural setting). *Notes: A = Public Primary School and Nursery1 Km away; B = Private Primary School and Nursery 1 Km away*

4.2.5 Sample size estimation

A previous study (Akosu and Zoakah, 2008) on risk factors associated with dental fluorosis in Central Plateau State, Nigeria, reported an overall dental fluorosis prevalence rate of 12.9%. In the current study, the sample size for the main study was calculated using a power of 95%, 90%, 85% and 80% at an alpha level of 5%, with a maximum difference of 3% between at least two of the 8 units (4 and 8 year olds, in rural and urban, low and high

water F areas) and standard deviation of 10% using Minitab 17 Statistical software (Minitab 17 Statistical Software (2010). [Computer software]. State College, PA: Minitab, Inc. (www.minitab.com).

The estimated sample size based on a power of 95% was 290 for each of 4 year-olds and 8 year-olds (Table 4.4). However, to allow for an expected attrition rate of 30%, the overall sample size was set at 616 (308 four year-olds and 308 eight year-olds).

Maximum Difference	Sample size	Target Power
3	290	0.95
3	235	0.90
3	201	0.85
3	176	0.80

Table 4-4: Power and sample size calculation.

4.2.6 Ethical considerations

4.2.6.1 Ethical approval

Ethical approval was obtained from the Ethical Review Committee at Newcastle University, UK (Appendix E) and University of Ibadan/University College Hospital, Nigeria Ethical Review Board (Appendix F) through submission of a detailed study protocol.

4.2.6.2 Permission from the Ministry of Education

Once a positive ethical approval had been received from Newcastle and Ibadan universities, approval to undertake the study in the selected nurseries and primary schools was obtained from the Nigerian Ministry of Education, Oyo State, Nigeria (Appendix G). The permission obtained included the carrying out of a dental examination and, taking of intraoral photographs and buccal swabs of the children. A brief protocol of the study was given to the Ministry of Education to ensure that they understood the details and were able to give permission for all aspects of the study.

4.2.6.3 Permission from the Local Government Education Board

Once permission had been received from the Nigerian Ministry of Education, verbal approval was obtained from the Local Government Education Board to visit the selected nurseries and primary schools and undertake the study. The brief study protocol were given to the board to ensure that all aspects of the study were permitted by their rules and regulations.

4.2.6.4 Permission from the selected nurseries and primary schools

Verbal approval to visit each of the selected nursery and primary school and collect demographic information from records was obtained from the head-teacher of each of the selected nursery and primary. This approval included collection of a 24 hour urine, height and weight measurement, and dental examination and buccal swab. Permission to unpack research a van and upload research materials and equipment on nursery and school premises were also obtained.

4.2.6.5 Permission from parents or legal guardians and 8 year olds

A letter (Appendix H) was written to parents or legal guardians of participants requesting for permission to obtain information about their socio-demographic/socio-economic background and child's dietary and tooth cleaning practices. This permission also included a request for demographic information such as age to be obtained from nursery and primary school records. The parents or legal guardians were requested to grant permission for their child's teeth to be examined, buccal mucosa to be swabbed and 24 hour urine to be collected. This letter was translated into Yoruba Language for parents or legal guardians who were illiterate or could not understand English. A participant information sheet (PIS) (Appendix I) translated into Yoruba was also sent to the parents or legal guardians, also translated into Yoruba Language for those who could not understand English. The participant information sheet contained an illustration of the dental examination and non-invasive swabbing of the inside of the cheek. A separate participant information sheet (Appendix J) providing information on collection of 24 hour urine was given to parents or guardians whose child participated in Phase 2 of the study. The invitation letter (Appendix H) sent to parents or legal guardians also invited them for a meeting which was held at a specified date when they came to collect their child/ward or during Parents Teachers Association meeting in the nursery or school premises. During this meeting, they were informed about the detail of the study. The meeting was interactive and answers to any questions asked were provided. Parents and legal guardians were asked to think through the study within 2 days before consenting to participate and allow their children to participate in the study.

The letters to parents or guardians contained a written consent form (Appendix K) in simple lay person English but also translated into the Yoruba language. The consent form contained a section requesting the children to assent to participate in the study. The documents were sent/distributed two weeks before the commencement of the study. The research assistant ensured that the consent form was signed (or thumb printed by parents or

legal guardians who were not educated) before their children or wards participated in the study. The participant information sheet (Appendix I and J) containing information regarding examination procedures, programme activities, and benefits expected from the study was mentioned in the letter. It also stated that a participant was free to withdraw at any time without any harm for doing so. Arrangement was also made for a school-centred debriefing after the end of the study.

4.2.7 Sampling technique

The study was designed and sampling was undertaken in a manner that aimed to minimise bias and the researcher was very mindful of potential sampling errors. Cluster sampling was used to recruit 4 and 8 year-olds from both public and private nurseries and primary schools respectively, based on United Nation Educational Scientific and Cultural Organization (UNESCO), 2010 data in which an enrolment ratio of 8:1 in Nigerian public and private schools respectively was stated. Based on this ratio, 269 and 39 four year-olds plus 269 and 39 eight year olds were planned to be randomly recruited from public and private nurseries respectively, with the schools as clusters, in the four study locations as shown in Figure 4.2 (See also, Section 5.3.2 – The sample). Nurseries and primary schools closest to the study area were identified from the local Government Secretariat (see section 4.2.3.2 and 4.2.3.3) list and they were visited one after the other until enough participants were randomly recruited into the study. The method of choosing nurseries and primary schools closest to study area was adopted to ensure homogeneity amongst participants. Phase 2 of the study was planned to be undertaken using a sub-sample of the Phase 1 participants and Phase 2 was reliant on children being in control of their bladder to facilitate valid 24h urine sample collection. Due to this requirement the same inclusion and exclusion criteria were used for both phases to recruit from the outset of the study.

4.2.8 Inclusion criteria

1. Males and females aged 4 and 8 years at the time of dental examination.

2. Healthy children of both genders with no health problems, including chronic metabolic and renal diseases.

3. Children who had not received professionally applied fluorides or dietary F supplements in the 3 months prior to recruitment phase of the study.

4. Children who were not bed-wetting.

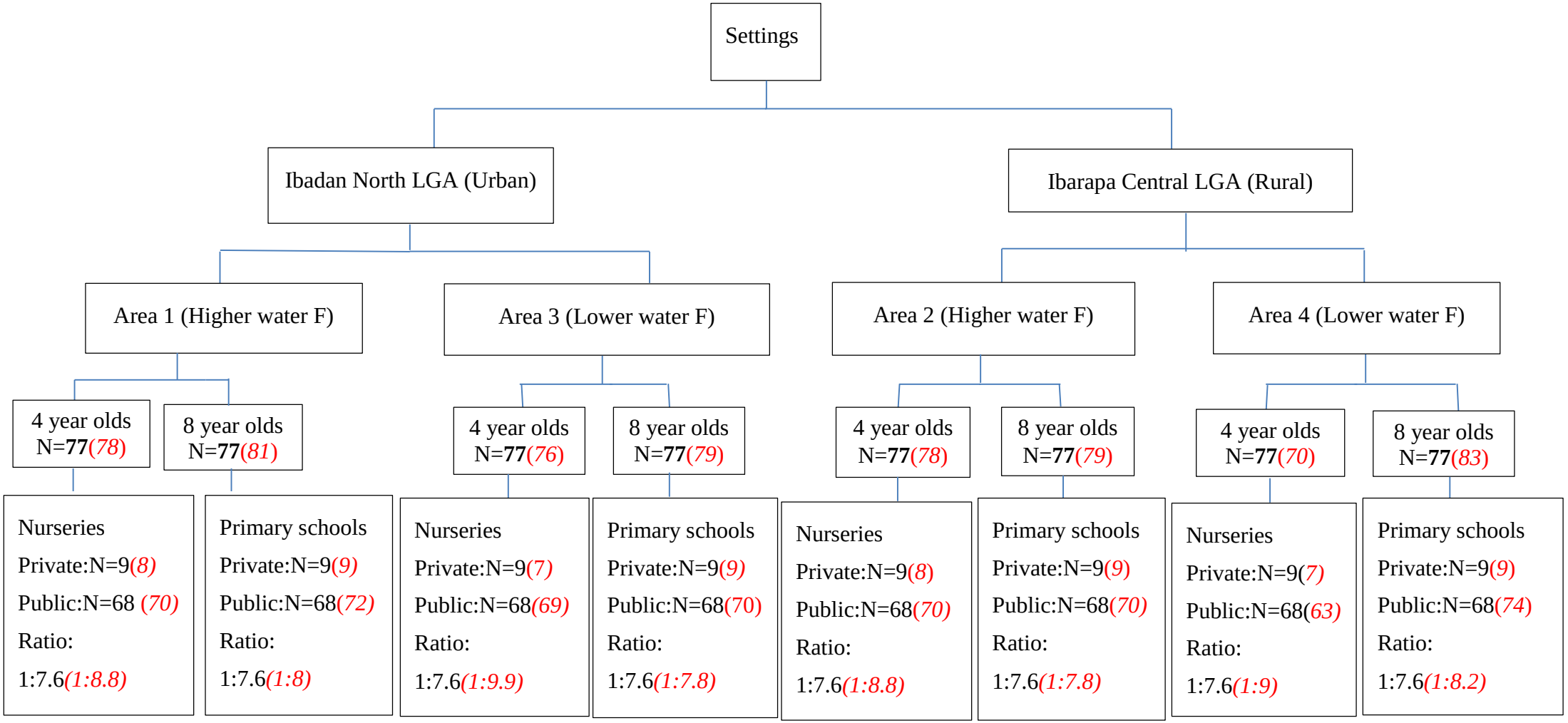

Figure 4-2: Planned and actual cluster sampling of study participants for Phase 1 from nurseries and primary schools in urban and rural higher (≥0.6 ppmF) and lower (≤0.2 ppmF) water F concentration in community water supply.

Notes: Planned numbers and ratios in black; Actual numbers and ratios - in red, italicised and in parentheses

4.2.9 Exclusion criteria

1. Children who were not aged 4 and 8 years at time of dental examination.

2. Children whose parents or guardians could not provide written informed consent

3. Children with an underlying medical problem, including chronic metabolic and renal diseases.

4. Children who had received dietary F supplements or professionally applied fluorides in the 3 months prior to recruitment phase of the study.

5. Children of families who planned to move from the area of residency during study.

6. Children suffering from bed-wetting. (In the event, no participant was excluded because of bed-wetting as all participants had full bladder control. See Section 6.4.1).

4.2.10 Preparation for the study

4.2.10.1 Preparation in the UK

The dental examination sheet, photographic log, questionnaires and urine data sheet were developed as described in Chapters 5 & 6 and the electronic forms were saved and backed-up. The researcher underwent training on clinical dental examination to measure Developmental Defects of Enamel and Dental Fluorosis by a trained examiner prior to the field work in Nigeria. Similarly, training on photographic image taking was also carried out to ensure that images of Dental Fluorosis were accurately taken. In addition, the researcher received laboratory training on analysis of drink, food and urine samples to i) measure F concentration of those samples using an F-ISE by direct and indirect method as appropriate and ii) to provide relevant training for a laboratory technician in Nigeria to analyse the collected urine and water samples from the study participants. A detailed Gantt chart (Appendix L) scheduling the field work in Nigeria and a delegation log for members of the research team was drawn up to ensure that the work was carried out efficiently and effectively. The essential materials used for the field work were procured via the Newcastle University procurement systems and where necessary from commercial shops. A Nikon digital camera was borrowed from the Borrow Foundation, UK and a stadiometer from the F research laboratory, Newcastle University. Authorization to transport buccal mucosa swab, food and drink samples was obtained from Department for Environment, Food and Rural Affairs (DEFRA) (Appendix M). The research materials were packed carefully and then transported from the UK to Nigeria through a courier company.

4.2.10.2 Preparation in Nigeria

Some materials, especially consumables, were procured from reputable commercial shops in Nigeria. Copies of the data collection sheet and other research documents were printed and photocopies were made. These documents were securely kept in a locked locker in the oral pathology laboratory of the University of Ibadan, Nigeria. The research materials transported from the UK to Nigeria were received intact without any damage and a research van was hired to ensure easy transport of members of the research team and materials, to and from nurseries and schools during fieldwork. Advertisements were placed on notice boards in the University of Ibadan and the University College Hospital Ibadan, Nigeria in forming the public and staff about the project and asking for those with relevant qualifications to apply for the post of laboratory technician, nutritionist, record clerk and research assistants to be employed as part of the project team. Applications were received and screened, after which interviews were conducted to select members of the research team.

The members of the research team who were successful in the interview were trained over a two week period just prior to the commencement of the study. They were informed about the aims and methodology of the study and a delegation log given to each of them to ensure that they knew their roles. Similarly, the work schedule was also provided to indicate the timeline for the various aspects of the study. They were encouraged to ask questions if certain issues needed clarification and were also informed of the need to maintain confidentiality of the data collected. To facilitate the recruitment of school staff, parents/legal guardians and their children/wards, head teachers' permission and support was necessary (Esbensen et al., 2008). Therefore, the head teachers of selected nurseries and primary schools were visited by the researcher and the record clerk before the commencement of the study. During the visit the letter requesting for permission to carry out the study was given to the head teacher and attached to this letter was the approval from the Nigerian Ministry of Education and a schedule for the data collection. The detail of the study was enclosed in this letter so that full approval could be granted. After obtaining approval to carry out the study in the selected nurseries and primary school, the date of birth of the pupils were checked in the attendance register for each class to identify those whose age met the inclusion criteria of the study. A letter inviting parents/legal guardians of children/wards, whose age met the inclusion criteria for the study, to a meeting in the nursery of school premises was sent (Appendix H).

The parents/legal guardians who responded to the invitation were met in a comfortable classroom provided by the nurseries and primary schools. Details of the study were explained and answers provided for any questions asked to ensure clarity. A list of study participants whose parents/legal guardians consented to allow them participate in the study was drawn up by the record clerk. On each field work day the members of the research team and research materials were transported to the selected nurseries and primary schools with a research van. Each time on arrival at the selected nursery or primary school, before the commencement of the field work, the head teacher was informed about the arrival of the research team, to take stock about level of work done and to solicit for continue support. After notifying the head teacher about the arrival of the team, research materials were taken out of the van and placed in the research room provided by the nursery or school authorities.

4.2.11 *Data collection*

The data were collected between January 2013 and June 2013. The following data were collected for Phases 1, 2 and 3.

Phase 1: a clinical dental examination was recorded on the examination sheet; photographic imaging was carried out using a digital camera and recorded in the log sheet and information on socio-economic status or parents or legal guardians of study participants, infant/childhood illnesses, feeding and tooth cleaning practices were collected using an interviewer-administered questionnaire. In addition, drinking and cooking water samples were collected from parents or legal guardians of study participants (see Chapter 5).

Phase 2: Information on food and drink consumption patterns was collected from parents and legal guardians of a subsample of those participating in Phase 1 of the study, using an interviewer-administered food frequency questionnaire. Food, drink and urine samples were collected from the Phase 2 study participants for the Phase 2. In addition, fluoride toothpastes recorded as commonly used by the study participants were also purchased from commercial shops (see Chapter 6).

Phase 3: a buccal mucosa swab was collected from the Phase 2 sub-sample of children using a non-invasive brush (see Chapter 7).

4.2.12 *Study steering group*

The researcher communicated with his supervisors via weekly emails and a skype meeting was held on two occasions. On one of these occasions, a technical problem involving the

presence of specular reflections on images of the teeth due to rays of light from perforated roofs, doors and windows was resolved with the suggestion that a black umbrella should be used to shield off the rays of light.

4.2.13 *Data management in Nigeria*

On a daily basis during the field and laboratory work, collation and verification of data for errors and omissions was carried out by manually checking the completed questionnaires, data sheets, field work diaries and laboratory books. The data collection documents and photographic images were kept securely in a locked locker in a locked room at the University of Ibadan. Any borrowed research materials and data collection documents were carefully packed and transported in the researcher's hand and hold luggage from Nigeria to the UK after the field work. The drink and food samples and buccal mucosa swabs were also carefully packed with dry ice and transported to the UK via a courier firm.

4.2.14 *Data management in the UK*

On arrival of the researcher in the UK, the questionnaires were coded and 10% were recoded to check for validity and reliability of the coding. After the genetic analysis (see Chapter 7) and F concentration measurement in food, drink and toothpaste samples using the F-ISE (see Chapter 6) data were entered into separate excel spreadsheets based on the category or type of data. Then 10% of the recoded data were re-entered into excel spreadsheets to check for validity and reliability of the coding and data entry. Data were cleaned electronically and summary statistics derived to detect unexpected and erroneous values.

4.2.15 *Data analysis*

Data were exported into Statistical Package for Social Science version 22 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp) after data cleaning. Frequencies, proportions, percentages, means and diagrams of relevant variables were generated. Percentage agreement and Cohen's kappa were used to determine intra-examiner's variability. The student t-test was used to compare means while chi-square test was used to test associations between categorical variables at the 5% level of significance. Correlation analysis was undertaken to determine relationships between relevant variables while regression analysis was used to predict the risk of occurrence of developmental defects of enamel and dental fluorosis.

4.3 Summary

This chapter described the pilot study undertaken in 2 stages in the urban (Ibadan North Local Government Area) and rural settings (Ibarapa Central Local Government Area) which provided information on the mean F concentration collected from wells and boreholes from these locations (Table 4.1). On this basis, wells and boreholes in 4 areas (Area 1-4) across the 2 Local Government Areas were selected and categorised as higher and lower water F based on their mean F concentration as shown in Table 4.5 and used as the settings for Phases 1-3 of the main study. An overview of the materials and methods for the main study was also described in this chapter to provide information by which the study validity was judged. The detail, including a clear and precise description of how the main study was conducted is provided later in the relevant chapters (5, 6 and 7) for the 3 Phases of the study; Phase 1 (Chapter 5 - Dental health and nutrition), Phase 2 (Chapetr 6 – Estimation of F exposure) and Phase 3 (Chapter 7 – Prevalence of Single Nucleotide Polymorphism).

Area	1	2	3	4
Urban/Rural	Urban (Ibadan North LGA)	Rural (Ibarapa Central LGA)	Urban (Ibadan North LGA)	Rural (Ibarapa Central LGA)
Ward	12 & 6	1 & 3	3	5
Higher/Lower water F	Higher	Higher	Lower	Lower
Mean F concentration of water in the Ward (mgF/L)	1.00	3.00	0.04 - 0.07	0.06
Water source	Borehole	Borehole/well	Borehole	Well

Table 4-5: Characteristics of the study locations by community water fluoride concentration (mgF/L).

Chapter 5 Phase 1 of the main study – Dental Health and Nutrition

5.1 Introduction

Morbidity rates such as incidence and prevalence of disease are essential indicators of public health and health care needs of a population (van Baal et al., 2011). They play an important role in measuring the burden of disease (Michaud et al., 2001, Lopez et al., 2006), making projections of future population health (Feenstra et al., 2001, Hoogenveen et al., 2009) and aiding policy decision making. Information about the distribution of developmental defects of enamel, dental fluorosis and dental caries helps to describe their current burden in the population to facilitate planning and resource allocation for both preventive and restorative services, while information on associated factors will help in understanding the aetiology of these conditions and disease.

5.2 Aim

To determine the prevalence and severity of developmental defects of enamel, dental fluorosis and dental caries and factors associated with their occurrence among 4 and 8 year olds in Nigeria.

5.3 Materials and methods

5.3.1 Study locations

As described in Chapter 4, the study was located in rural and urban areas of high and low water fluoride concentrations of south western Nigeria. The study locations in the urban community were Wards 12 and 3 in Ibadan North LGA representing higher (>0.6 ppmF) and lower (<0.2 ppmF) water fluoride areas respectively. In the rural community, Wards 1 and 5 in Ibarapa Central LGA representing higher (>0.6 ppmF) and low (0.02 ppmF) water fluoride areas respectively were the study locations, being selected based on the results of the pilot study described in Chapter 4.

5.3.2 The sample

Following ethical and confidentiality approval in the selected communities, male and female children aged 4 and 8 years residing and living within walking distances of their nurseries and primary schools were invited to participate in the study. As described in Chapter 4, cluster sampling of children or wards whose respective parents or legal guardians consented to their participation was used to randomly recruit study participants for Phase 1 of the study using public and private nurseries and primary schools as clusters

as shown in Figure 4.2 which includes the actual number of children recruited. One public and one private nursery and primary school nearest to the centre of the study location were first visited to randomly recruit study participants, however data was collected from them on first come first recruit basis. Then the next nearest nurseries and primary schools were visited in each of the 4 locations, participants recruited and valid informed consent obtained until the sample for all 4 areas was complete.

5.3.3 Preparation for data collection

5.3.3.1 Preparation in the UK

Development of data collection documents and training in their use: A clinical dental examination sheet (Appendix N) for recording dental caries, developmental defects of enamel and dental fluorosis was developed based on the WHO guidance for oral health surveys (WHO, 1997). Similarly, an interviewer-administered questionnaire used to obtain information on socio-economic status of parents or legal guardians, infant/childhood illnesses, feeding and tooth cleaning practices (Appendix O), was developed. This questionnaire was translated into Yoruba, the local language for non-English speakers. After translation, the questionnaire was back-translated into English and compared with the original to ensure consistency. The questionnaire was examined for face validity, operationalization and language. A photographic log book for recording images of the teeth was also developed.

Training of the researcher (OI):

Clinical dental examination: Three day training on how to measure and record dental caries, developmental defects of enamel and dental fluorosis was conducted by a trained examiner. During this training photographic digital images of teeth with or without dental caries and developmental enamel defects were examined electronically and on paper using the relevant indices. Intra- and inter-examiner reproducibility was calculated as greater than 90%.

Photographic imaging of the teeth: Training on how to set up a Nikon D90 single-lens reflex (SLR) digital camera with a Sigma 70mm 1:2.8 DG Macro lens and a Sigma Electronic FlashMacro EM-140 DG flashgun was carried out by a trained examiner (Dr Michael McGrady, University of Manchester). This training also included how to take quality images and keep good field records of images taken. During this training, images of

the teeth of a child who volunteered (son of a supervisor) were taken and recorded on the photographic log. The researcher was calibrated after the two training sessions.

Fluoride analysis: A trained postdoctoral researcher provided refresher F analysis training for water samples using a direct method and Fluoride Ion Selective Electrode (F-ISE).

Procurement and transport of research materials from UK to Nigeria: The Nikon digital camera, macro lens and flashgun were provided on loan by the Borrow Foundation, UK while the plastic pipettes, dispenser, TISAB, Fluoride standard solution, disposable dental mirrors, handle with a light source and cheek retractors were procured via the usual university procurement systems and where necessary from shops in the UK. Other materials such as stadiometer and digital weighing scale were borrowed from the Fluoride Research Team store at Newcastle University. These materials were transported together with other materials used for Phase 2 and 3 of the study to Nigeria via a courier company.

Scheduling of the study: A detailed Gantt chart (Appendix L) of the 6 months of fieldwork activities in Phase 1 was compiled, based on the stepwise activities of this phase of the study and the time anticipated to carry them out.

5.3.3.2 Preparation in Nigeria

Visit to the Head of Dental School: The researcher (OI) paid a courtesy visit to the Head of the Dental School University of Ibadan, Nigeria, where the researcher works to inform him about the field work and to confirm their support for the study.

Securing laboratory (including freezer storage) space: Written confirmation of the verbal approval received for a laboratory space including freezer storage during the pilot study was obtained.

Procurement of research materials in Nigeria: Research materials such as a wooden dental chair, wooden mouth spatulas, gauze, cotton wool, gloves, stool, plastic bowls, dark goggles and umbrella were procured in Nigeria. Printing and photocopying of the clinical examination sheet and questionnaires was also carried out.

Recruitment of members of the research team: A record clerk who helped to record names of study participants, follow-up participants from their classrooms to the research room and record clinical findings was recruited. Also, a nutritionist who administered the questionnaires and distributed drinking and cooking water collectibles to parents or legal guardians of participants was recruited. Furthermore, a laboratory technician was recruited to measure F concentrations in the water samples brought by parents or legal guardians.

Training of other research team members: The record clerk underwent a 2 day training which included how to record names and ages of study participants in the field-work log, identifying recruited study participants in their classrooms and guiding them to the research room, assisting them to brush their teeth with fluoride toothpaste, completing the clinical dental examination sheet and assisting the researcher (OI) during intra-oral image taking. As part of the training, the record clerk demonstrated these tasks on 4 volunteers. The nutritionist was trained by the researcher (OI) on how to administer the questionnaires to the parents or legal guardians of the study participants. After training, the nutritionist administered the questionnaires to 5 volunteers and any observable errors were corrected. In addition, the nutritionist was trained on how to label the water collectibles with the participant's identification number and type of water. The laboratory technician was trained over 2 days on how to measure F concentration in water using a F- Ion Selective Electrode and the direct method. This training was done using the F analysis training manual (Omid et al., 2011) and prior to the training; the technician was given the manual to read.

5.3.4 Data collection

Maintaining the schedule of the study: The schedule of work was checked after each day's work to monitor number of study participants recruited and if study was going as expected.

Transport of research team and materials to study location: The research van provided by the researcher was used to transport research team members and materials to the participating nurseries and primary schools. All materials were checked to ensure they met the daily need and were in good condition before they were transported to the study locations.

Validity and reliability of the measuring devices: As part of the preparation for the research, a pressing iron weighing 2.5kg and the procured stool which was0.6 metres in height were measured to calibrate the weighing scale and stadiometer respectively.

Setting up: At arrival at each selected nursery or primary school, before the commencement of the field work, the head teacher was visited to inform him/her about the arrival of the research team, to take stock about the current level of work done and to confirm continued support to complete the data collection. The items for the intra-oral images such as the digital camera, calibration cards, mouth props, sun glasses, stool and gloves were placed in a dark corner of the designated research room to decrease the intensity of sunlight. Items for the clinical dental examination such as dental chair,

examination table, chairs for the examiner and record clerk and examination set were placed close to the window so that vision could be enhanced. A table and questionnaires were also positioned in a suitable section of the room to facilitate the nutritionist administering the questionnaires.

5.3.4.1 The Questionnaire

Parents and guardians who agreed that child should participate were interviewed by the nutritionist using a slightly modified semi-structured interviewer-administered questionnaire (Appendix O). The questionnaire sought the following information for each child; level of parental or guardian education and income, length of residence in the community as well as information on their health, feeding and teeth cleaning practices during infancy and childhood.

5.3.4.2 Clinical dental examination of the teeth

The room designated in each school as the research room was prepared at the start of each session by the research team. After tooth brushing, the participants were dentally examined on an adjustable, collapsible and high backrest wooden chair to which a portable head rest was attached as shown in Appendix P. The examination to record teeth present, dental caries, developmental defects of enamel (DDE), and dental fluorosis (DF) was undertaken using the clinical dental examination sheet. The examination was carried out using a wooden spatula and an oral examination device comprising a disposable mouth mirror attached to a handle (DenLite Illuminated Dental Mirror, Miltex Inc. USA). This device also has a light source (white colour spectrum) that illuminates the oral cavity for clear examination of the teeth. The examiner and record clerk sat on either side of the chair. The illustration of the dental examination in the participant information sheet (Appendix I) was shown to the participants before the procedures commenced. The children were informed that the examination would only involve examining their teeth with dental mirrors and taking photographs of their teeth. They were familiarised with the procedure by being encouraged to try and do the dental examination themselves in-front of a standing mirror. Dental plaque was removed with dental gauze if it covered the tooth surface prior to the dental examination. The following assessments were made:

Teeth Present and Dental Caries Assessment

All the surfaces of all the primary and permanent teeth present in the mouth were examined systematically, from upper right to upper left quadrant and then from lower left to lower right quadrant. Incisal edge caries or restorations of anterior teeth were classified as labial

surface caries or restoration. Cuspal caries or restorations of molars were classified as occlusal surface caries or restorations. A tooth was classified as erupted when the enamel had become exposed to oral fluids. The presence of supernumerary or supplemental teeth was also noted on the examination chart. Dental caries was recorded clinically using the DMFT/dmft index shown in Appendix Q.

Assessment of Developmental Defects of Enamel (DDE)

Developmental defects of enamel (DDE) were recorded clinically using the DDE index as modified by Clarkson (1989) and FDI (1992b). The buccal surfaces of ten index teeth were examined; for any missing index teeth the corresponding cell in the examination chart was left blank. Buccal surfaces from the incisal edges or cuspal points to the gingiva and from mesial to the distal embrasure were inspected visually for defects. Any doubtful areas such as hypoplastic pits were checked with a periodontal probe to confirm the diagnosis. The type of DDE on each buccal surface was recorded as described in Appendix Q.

Assessment of Dental Fluorosis

Dental Fluorosis was recorded using Dean (1942) and Thylstrup (1978) fluorosis indices. The recording of the Dean's index was made on the basis of the two teeth that were worst affected using the criteria in Appendix 5.5. If these two teeth were not equally affected, the score for the less affected of the two was recorded. When the teeth were scored, the higher end of the index i.e. "severe" was first considered and each score was eliminated until the present condition was arrived at. If there was any doubt, the lower score was given. The TFI recording was made for the buccal surface of each tooth present using the criteria described in Appendix Q.

Photographic dental examination of teeth

The buccal surfaces of anterior and posterior teeth were photographed with a Nikon D90 single-lens reflex (SLR) digital camera with a Sigma 70mm 1:2.8 DG Macro lens and a Sigma Electronic FlashMacro EM-140 DG flashgun using only the upper illumination element anteriorly and laterally. The Standard Operating Procedure developed for use during the training was followed throughout the data collection process. Before the images of the participant's teeth were taken, an image of the participant's identification number (area and school code and participant' study number) attached to the collar of his or her clothes was first taken so ensure easy identification of images of the teeth and all images were checked for clarity before moving on. Where there were lip shadows or specular

reflections, images were deleted. A photographic log form was completed linking digital files to the unique subject identifier as shown in Table 5.1.

Serial number	Date	Area and School Code	Study number ID	Number of images	DSC numbers

Table 5-1: Photographic log of images taken.

Each day, 18 children were dentally examined and images of their anterior teeth taken. Two children were also randomly selected for a second dental exam by balloting from the list of the 18 children and dentally re-examined for reproducibility testing. The worse DDE mouth score was used for the intra-examiner reproducibility.

5.3.4.3 Drinking and cooking water samples collection and analysis

After the nutritionist administered the questionnaire, parents or legal guardians were given two clearly labelled 10 ml universal bottles and asked to collect and return a current customary drinking and cooking water sample used by their child/ward and these samples were then analysed for F concentration. Parents or legal guardians who were not able to provide the water samples before the research team left the study location that same day brought them the following day. The water samples were taken daily to the Genetics Laboratory, Institute of Medical Research and Training, University of Ibadan, Nigeria for F concentration measurement. The F concentrations in the water samples were measured using the F Ion Selective Electrode by a direct method by the laboratory technician using the F analysis training manual (Omid et al., 2011) developed for this method (Martínez-Mier et al., 2011) and refined for use in Nigeria during the training period. Water samples were disposed of in the laboratory sink immediately after F concentration measurement. The water F concentration (ppm F) was classified into 3 groups namely Lower (<0.7), moderate (0.7-1.2) and higher (>1.2).

5.3.4.4 Transport of data collection documents and camera together with images of the teeth

The dental examination sheets, questionnaires, laboratory book and digital camera together with the images of the teeth were carefully packed and transported appropriately in the researcher's hand or hold luggage from Nigeria to the UK.

5.3.5 Data and samples management and handling in Nigeria

The completed questionnaires were collated and verified for errors and omissions by checking the questionnaires daily after the field work. Where there were errors and omissions, the parents or legal guardians were contacted and accurate responses were

recorded. The subject identification number on the dental examination sheet was checked against questionnaires and field work dairy. Each digital image of the teeth was checked for specular reflections, lip shadow and clarity immediately after the intraoral photograph. Where there were errors, the photographs were retaken before the study participants left the research room. At the end of the field work each day, the images were re-checked for errors and omissions and if necessary, the study participants were identified and the photograph retaken. The photographic log book was also checked for errors and omissions.

For all drinking and cooking water samples collected, the F analysis recordings in the laboratory book were checked regularly for errors and omissions. The questionnaires, clinical dental examination sheets, photographic log book and filled laboratory book were photocopied and the photocopies were kept in a locked locker in a locked room at the Dental School University of Ibadan, Nigeria. Similarly, the photographic images of the teeth were copied from the camera memory card into an external drive and two memory sticks. All paper based data were photocopied and the photocopies were kept safely in a locked filing cabinet in Nigeria as a back-up and the originals transported to the UK in the researcher's hand and hold luggage during the travel from Nigeria to the UK.

5.3.6 Data and samples management and handling in the UK

Dental health and Nutrition questionnaires: On arrival in the UK, a coding sheet was produced (Appendix R). Prior to this, an independent researcher randomly selected 10% of the questionnaires and photocopies of these questionnaires were made. The responses for all questionnaires were transferred into coding sheets which were sent to a data entry firm for double entry in an excel spread sheet. After double entering, the data were checked for errors and omissions against the coding sheets and the original questionnaires. The responses in the photocopied questionnaires (10% of original sample) were also checked against their original to confirm validity and reliability of the recoding.

Clinical dental examination data: The data were entered into an Excel spreadsheet and checked for errors and omissions. The data were read from the Excel spreadsheet using the AWK programming language (Aho et al., 1988). The dmft/DMFT, DDE and TFI indices were calculated and the data exported in a format suitable for reading into the SPSS statistical package.

Photographic imaging of the teeth: On arrival in the UK, the digital images were sorted out by checking the study number ID comprising the area code, school code and study participants' ID and the DSC numbers of the images in the photographic log book against

their images in the external drive. The image of the study participant's ID was removed to ensure that the independent examiner (MGM), who graded the teeth seen in each image using the same TF index used for the clinical examination, was unaware of the locality of the subject. All images were exported from the external drive and integrated into a graphical user interface on a computer by the examiner (Dr M McGrady). The images were viewed on a 32-inch screen at 5x magnification after they were randomized and blinded by the graphical interface. Images of the maxillary central incisors were scored for dental fluorosis using the TF index by the examiner (Dr M McGrady). For each study participant, the highest TF score recorded for either maxillary central incisor was the value recorded. Images which did not clearly show all the buccal surface of a tooth were not scored.

Drinking and cooking water samples data: The accuracy and validity of the fluoride analysis by the direct method was estimated by re-analysis of 7% of the water samples. The data were entered into excel spreadsheet and 10% of the data were randomly selected by an independent researcher and re-entered into another spreadsheet to check for validity and reliability of the data entering. The data were printed out and their print out compared but were no difference.

5.3.7 Data analysis

Data were entered into Statistical Package of Social Sciences (SPSS) software for descriptive and statistical analysis. Data were checked for normality; for normally and non-normally distributed data parametric and non-parametric analyses were conducted respectively. Validation and reproducibility of dental examination data was carried out using the worst DDE score for both DDE recordings of the 10% of the study participants that were re-examined and these scores were compared using kappa statistics. Descriptive analysis was presented as proportions and mean (SD) for parametric data while proportions and median (range) were used to describe non-parametric data. One way ANOVA and Tukey Post Hoc Tests where relevant were used to examine for statistical significance differences in the mean F concentrations in drinking and cooking water according to study areas, age and gender at 5% level of significance. Correlation analysis was carried out to determine the direction and strength of the relationships between relevant variables at $p<0.05$.

5.4 Results

5.4.1 Duration of data collection and recruitment of study participants

The data collection was carried out between January and June 2013 in public and private nurseries and primary schools and 624 study participants comprising 302 four year old and 322 eight year old participants were recruited into Phase 1 of the study (Table 5.2). The planned ratio of recruitment for private and public nurseries and private schools was 1:7.6 while the actual ratio of recruitment was within the range of 1:7.8 – 1:9.9.

5.4.2 Validation, verification and reproducibility of data:

5.4.2.1 Questionnaires

A validated questionnaire from the Iowa Fluoride Study was adopted and used for the study. Prior to its use, the questionnaire was modified to suit the research setting by removing sections on fluoride and vitamins supplements and changing toothpaste names to those of present setting. Questions on types of foods and drinks were also changed to open ended questions so as to explore the types consumed in present setting. The questionnaires were translated into local language and back-translated into English language by an expert in both English and local language.

5.4.2.2 Dental examination data

The result of the kappa statistics for the 10% of the study participants is as shown in Table 5.3. The measure of agreement for re-examination of DDE was 0.963, 0.961 and 0.892 for the 4 years with primary teeth, 8 years with primary teeth and 8 years with permanent teeth respectively and these agreements were statistically significant ($p<0.001$).

5.4.2.3 Digital image

The result of the kappa statistics for 20% of the study participants whose digital images were re-scored is as shown in Table 5.4. The measure of agreement was 0.828 and these agreements were statistically significant ($p<0.001$).

5.4.2.4 Fluoride analysis data

The result of the kappa statistics for the 7% drinking and cooking water samples is as shown in Table 5.5. There was no statistically significant difference between the mean (SD) first and repeat F concentration measurements in the drinking and cooking water at $p=1.00$ and $p=0.95$ respectively. The measure of agreement was 0.819 and 0.798 for the drinking and cooking water respectively.

Data collection and recruitment	Area 1 (Urban, Higher F) (n=159)				Area 2 (Rural, Higher F) (n=157)				Area 3 (Urban, Lower F) (n=155)				Area 4 (Rural, Lower F) (n=153)				Overall Total (n=624)			
Duration of data collection	21/01/13 to 22/02/13				05/03/13 to 05/04/13				22/04/13 to 24/05/13				27/05/13 to 28/06/13							
Age (years)	4 years		8 years		4 years		8 years		4 years		8 years		4 years		8 years		4 years		8 years	
Study location	A		B		A		B		A		B		A	B	A	B	A		B	
Type of study location	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D
No. of Nurseries/primary schools	5	1	5	1	6	1	5	1	7	1	3	1	3	1	3	1	21	4	16	4
No. of study participants recruited	70	8	72	9	70	8	70	9	69	7	70	9	63	7	74	9	272	30	286	36
Total No. of study participants recruited	78		81		78		79		76		79		70		83		302		322	

Table 5-2: Duration of data collection, recruitment of 4- and 8-y old study participants in Phase 1 of the study (n=624). *Notes: A – Nurseries; B – Primary schools; C – Public and D – Private.*

Measure of agreement	4 years (Primary teeth) (n=36)			8 years (Primary teeth) (n=37)			8 years (Permanent teeth) (n=37)		
	N	Value	P	n	Value	P	n	value	p
	36	0.963	<0.001	37	0.961	<0.001	37	0.892	<0.001

Table 5-3: Measure of agreement for 10% of 4 and 8 year old study participants re-examined for DDE.

Measure of agreement	N	Value	p
	127	0.828	p<0.001

Table 5-4: Measurement of agreement for 20% of 4 and 8 year old study participants whose digital images of anterior teeth were re-scored.

5.4.3 Dental Health and Nutrition - Four year olds

5.4.3.1 Mean (SD) and Median (minimum, maximum) of fluoride concentration (mgF/L) in drinking and cooking water samples

The mean(SD) and median (minimum, maximum) of fluoride concentration (mgF/L) in drinking and cooking water of the 4-year old participants was lowest in Area 4 and highest in Area 2 and the differences between areas were statistically significant ($p<0.05$) (Table 5.6). The F concentration of the drinking water sample of 60 (19.9%) four year-olds had the same F concentration as their cooking water sample.

5.4.3.2 Socio-demographic data

The mean (SD) age ranged from 4.43(0.22) years in Area 4 to 4.55(0.25) years in Area 1 and the difference in age between children in Areas 1 and 4 was statistically significant ($p=0.01$), although the distribution of males and females in the 4 Areas did not differ significantly ($p=0.21$) (Appendix U). As Appendix V shows the majority 212(70.2%) of the 4 year olds had lived in their respective areas from birth while 90(29.8%) had lived in the areas for a shorter period. The urban populations (Areas 1 and 3) showed much less residency from birth for both males and females, while in the rural areas (Areas 2 and 4), the majority (96.2% and 74.1% respectively) of children had lived there from birth and these differences were statistically significant ($p<0.001$). Appendix W shows that the majority of the parents/legal guardians 231 (76.5%) reported receipt of one form of education, 286 (94.7%) engaged in one form of occupation and 200(66.2%) earned less than N10, 000 monthly. Seventeen (5.6%) reported that they earned no money at the end of the month or refused to mention what they earned monthly.

Measure of agreement	N	Measure of agreement	Mean (SD)	p
F concentration drinking water (First)	44	0.819	0.20 (0.17)	1.00
F concentration drinking water (Repeat)	44		0.20 (0.17)	
F concentration cooking water (First)	44	0.798	0.20 (0.19)	0.95
F concentration cooking water (Repeat)	44		0.20 (0.19)	

Table 5-5: Measurement of agreement for 7% of drinking and cooking water samples.

Water samples	Area 1 (Urban, Higher F) (n=78)	Area 2 (Rural, Higher F) (n=78)	Area 3 (Urban, Lower F) (n=76)	Area 4 (Rural, Lower F) (n=70)	All Areas (n=302)	p value*	Tukey Post-hoc
Drinking water samples							1v2=0.01
No. where F <0.1 mgF/l[a]	59	17	65	42	183	<0.001	2v4=p<0.001
No. where not measured[b]	3	0	5	1	9		
No. where F ≥0.1mgF/l	16	61	6	27	110		
Mean (SD) F conc. (mgF/l)	0.35(0.31)	1.10(1.04)	0.53(0.74)	0.25(0.15)	0.76(0.90)		
Median (Min, Max) F conc.(mgF/l)	0.25(0.10, 1.00)	0.80(0.20, 4.00)	0.20(0.10, 2.00)	0.20(0.10, 0.60)	0.40(0.10, 4.00)		
Cooking water samples							1v2=0.001
No. where F <0.1 mgF/l[a]	52	18	59	44	173	<0.001	2v3=0.01
No. where F not measured[b]	3	0	5	1	9		2v4=p<0.001
No. where F ≥0.1mgF/l	23	60	12	25	120		
Mean (SD) F conc. (mgF/l)	0.35(0.23)	1.10(0.99)	0.31(0.54)	0.29(0.16)	0.69(0.82)		
Median (Min, Max) F conc.(mgF/l)	0.30(0.1, 1.00)	0.80(0.2, 4.00)	0.10(0.1, 2.00)	0.20(0.10, 0.60)	0.40(0.10, 4.00)		

Table 5-6: Mean (SD) and median (minimum, maximum) of fluoride concentration (mg/L) in drinking and cooking water samples for 302 4-year old participants by area. Notes: [a] - *low F concentration (<0.1ppmF) below the detection limit of F-ISE*; [b] - *Not measured = Sample not collected*; * - *One way ANOVA*

5.4.3.3 Prevalence and severity of Developmental Defects of Enamel (DDE)

Mouth prevalence

Table 5.7 summarises the key dental health characteristics of the 4 year old participants, while more detailed results are contained in the Appendices. The prevalence of DDE varied between areas from 93.6% of children examined in Area 1 to 51.4% in Area 4. There was a statistically significant difference in the prevalence of DDE between the 4 Areas ($p<0.05$). Appendix X shows that more males (38 representing 50.0% of the 76 children examined) in Area 3 and females (39 representing 50.0% of the 78 children examined) in Area 1 had developmental defects of enamel, but overall the difference in the prevalence of DDE between males and females was not statistically significant ($p=0.51$). Appendix Y shows that across the 4 areas, diffuse opacities were the most prevalent type of DDE, ranging from 28.6% in Area 4 to 93.6% in Area 1.

Tooth prevalence

Table 5.7 presents the overall summary of the mean (SD) number of primary teeth affected by DDE by area. Overall, the mean (SD) number of teeth affected ranged from 1.39(1.85) in Area 4 to 6.14(3.25) in Area 1 and this difference was statistically significant ($p<0.001$). Appendix Z shows a statistically significant difference in the mean (SD) number of primary teeth affected by DDE among males and females between the 4 areas ($p<0.001$) except between Areas 2 and 3 ($p=0.17$); males and females in Area 1 had significantly more affected teeth ($p<0.001$). Overall, of the 1202 index teeth with defects, which represented 40.3% of the 2985 primary index teeth examined in the 302 four year olds, 58% involved diffuse opacities (Appendix AA).

The frequency distribution of the 1202 primary index teeth with DDE according to tooth type is described in Table 5.8. Of the primary index teeth examined, the upper right (14.6% of the total teeth affected) and left (14.2%) first primary molars were the primary index teeth mostly affected while upper left lateral (70 (5.9%)) and right lateral (83 (7.0%)) incisors were least affected.

DDE, Dental fluorosis and Dental caries	Area 1 (Urban, Higher F) n=78	Area 2 (Rural, Higher F) n = 78	Area 3 (Urban, Lower F) n = 76	Area 4 (Rural, Lower F) n = 70	All areas n = 302	P values	Post-Hoc Test
DDE							
Mouth prevalence (DDE > 0) No. (%)	73 (93.6)	66 (84.6)	60 (78.9)	36 (51.4)	235 (77.8)	< 0.001[+]	
Tooth prevalence (No. of teeth affected) Mean (SD)	6.14 (3.25)	4.64 (3.25)	3.55 (2.85)	1.39 (1.85)	4.00 (3.33)	<0.001[#]	1v2[*]; 1v3[*]; 2v4[**]; 1v4[**]; 3v4[**]
Dental fluorosis							
Mouth prevalence (Deans index) No. (%)	12 (15.4)	9 (11.5)	0 (0)	0 (0)	21 (7.0)	<0.001[+]	
Mouth prevalence (Direct clinical examination TFI > 0) No. (%)	9 (11.5)	8 (10.3)	0 (0)	0 (0)	17 (5.6)	<0.001[+]	
Mouth prevalence (Digital photographic TFI > 0) No. (%)	4 (5.1)	1 (1.3)	1 (1.3)	1 (1.4)	7 (2.3)	0.31[+]	
Tooth prevalence (No. of teeth affected) Mean (SD)	0.40 (1.40)	0.54 (2.00)	0 (0)	0 (0)	0.24 (1.26)	0.01[#]	2v3(p=0.04); 2v4(p=0.04)
Dental caries							
Mouth prevalence (dmft > 0) No. (%)	14 (17.9)	3 (3.8)	10 (13.2)	5 (7.1)	32 (10.6)	0.02[+]	
Tooth prevalence (No. of teeth affected) Mean (SD)	0.54 (1.43)	0.06 (0.34)	0.34 (1.08)	0.23 (1.09)	0.29 (1.07)	0.04[#]	1v2(p=0.03)

Table 5-7: **Summary of prevalence of DDE, Dental Fluorosis and Dental Caries in primary teeth of 4 year-old participants by area.**

Notes: [+] *- Chi-square;* [#] *- One way ANOVA;* [*] *- p=0.01; ** - p=0.001*

Primary teeth	No.	(%)
Upper right first primary molar	175	14.6
Upper left first primary molar	170	14.2
Upper right canine	154	12.7
Upper left Canine	151	12.5
Upper right lateral incisor	83	7.0
Upper left lateral incisor	70	5.9
Upper right central incisor	96	8.0
Upper left central incisor	91	7.6
Lower left second primary molar	104	8.6
Lower right second primary molar	108	8.9
Total	**1202**	**100.0**

Table 5-8: Frequency distribution of the 1202 primary index teeth with Developmental Defects of Enamel among 4 year-old participants with DDE according to tooth type.

5.4.3.4 Prevalence and severity of Dental Fluorosis

Mouth prevalence of Dental Fluorosis

The overall summary in Table 5.7 shows that, when Dean's and TF indices were used to assess teeth, only participants in Areas 1 (15.4% and 11.5%) and 2 (11.5% and 10.3%) had dental fluorosis in primary teeth. Appendices AB and AC show no statistically significant difference in the mouth prevalence of dental fluorosis between males and females ($p>0.05$). When dental fluorosis was assessed using digital photographic imaging of the upper primary central incisors, there was no statistically significant difference in the mouth prevalence among males and females across the 4 areas ($p>0.05$) (Appendix AD), but the recorded mouth prevalence rates were lower, ranging from 5.1% for Area 1 to 1.3% for Areas 2 and 3.

Tooth prevalence of Dental Fluorosis

Table 5.7 also presents the summary of the mean (SD) number of primary teeth affected by Dental Fluorosis by area. Overall, the mean (SD) number of primary teeth affected by dental fluorosis was statistically significantly higher in Area 2 (0.54(2.00) compared with Areas 3 and 4 (both 0) ($p=0.01$). Appendix AE shows no statistically significant gender difference in the mean (SD) number of primary teeth affected by dental fluorosis ($p>0.64$).

5.4.3.5 Prevalence and severity of caries experience among 4 year old study participants

Mouth prevalence of caries experience

As described in Table 5.7, 14 (17.9% of total examined) and 10 (13.2%) participants in the Urban Areas 1 and 3 respectively had caries experience while only 3 (3.8%) and 10 (13.2%) four year olds in the rural Areas 2 and 4 respectively had caries experience and this difference was statistically significant (p=0.02). As Appendix AF shows, there was no statistically significant difference in the mouth prevalence of caries experience between males and females (p = 0.36).

Tooth prevalence of caries experience

Table 5.7 shows that overall, the mean (SD) number of primary teeth affected by dental caries ranged from 0.06(0.34) in Area 2 to 0.54(1.43) in Area 1 (p=0.04). In addition, as Appendix AG describes, the mean(SD) number of primary teeth affected by dental caries ranged from 0.05(0.32) in Area 2 to 0.88(1.90) in Area 1 among males (p=0.01) while it ranged from 0.08(0.36) in Area 2 to 0.37(1.50) in Area 4 among females (p=0.51). There was no statistically significant difference in the mean (SD) number of primary teeth affected by dental caries between males and females (p=0.22).

5.4.3.6 Health during infancy and childhood

Ten (3.3%) parents or legal guardians reported that the birth of their child or ward was not normal. Whooping cough (20%), diarrhoea (18.9%) and measles (15.2%) were the main illnesses reported during infancy or childhood while 1 parent or legal guardian report that the child or ward had recurrent viral infections (Table 5.9).

Table 5.10 shows the age at which 4 year old participants reported having had whooping cough, diarrhoea and measles. The majority (28(45.9%)) who had whooping cough had it when they were 6 months of age or younger, while 17(29.8%) who reported diarrhoea had it when they were between 12 to 18 months old and 60.9% of those 4 year olds who reported a history of measles had suffered this when they were >18 months of age.

Twenty five (8.3%) parents or legal guardians reported that their child/wards' family had discoloured teeth; 11(44.0%) and 14(56.0%) of them stating that it affected paternal and maternal family members respectively. Two (0.7%) parents or legal guardians mentioned that other siblings had discoloured teeth.

Type of infancy or childhood illnesses	No.	(%)
Whooping cough	61	20.2
Diarrhoea	57	18.9
Measles	46	15.2
Pneumonia	14	4.6
Chicken pox	14	4.6
Trauma to face	12	4.0
Trauma to teeth	5	1.7
Rheumatic fever	4	1.3
Recurrent viral infections	1	0.3
Neonatal tetanus	1	0.3
Total with illnesses reported	**138**	**45.7**
Total with no illnesses reported	164	54.3
Overall Total	**302**	**100.0**

Table 5-9: Reported infancy or childhood illnesses among 4 year old study participants (n=302[1]). *Note: [1] - Multiple responses*

Age of having illnesses	Whooping cough		Diarrhoea		Measles	
	No.	(%)	No.	(%)	No.	(%)
≤6 months	28	45.9	12	21.1	3	6.5
7-11 months	7	11.5	16	28.0	0	0
12-18 months	20	32.8	17	29.8	15	32.6
>18 months	6	9.8	12	21.1	28	60.9
Total	**61**	**100.0**	**57**	**100.0**	**46**	**100.0**

Table 5-10: Age at which 4 year old study participants had whooping cough (n=61), diarrhoea (n=57), and measles (n=46).

5.4.3.7 Infant and childhood feeding practices of 4 year old study participants

Two hundred and fifty two (83.4%) parents and legal guardians reported that study participants were exclusively breast fed while 1 child (0.4%) was exclusively formula fed (Table 5.11).

Feeding practices	No.	%
Exclusive breast feeding	252	83.4
Mixed feeding	49	16.2
Exclusive formula feeding	1	0.4
Total	**302**	**100.0**

Table 5-11: Parents or guardians' self-reported early feeding practices of 4 year old study participants (n=302).

Prolonged breastfeeding appeared to be common to all areas as Table 5.12 shows, the majority 243(80.5%) of parents or legal guardians reported that their child/ward had stopped breastfeeding after 12 months of age.

Age	No.	%
< 2 months	1	0.3
2 – 5 months	8	2.6
6 – 11 months	50	16.6
≥ 12 months	243	80.5
Total	**322**	**100.0**

Table 5-12: Age 4 year old participants stopped breastfeeding.

With regard to parents/legal guardians' self-reported weaning foods and drinks and those currently consumed by 4 year old study participants, water, liquid milk or yoghurt and tea were the drinks mostly consumed during weaning by 300(99.3%), 128(42.4%) and 119(39.4%) of participants respectively (Table 5.13). However, the most popular currently consumed drinks were water, carbonated drinks and sugared-ready to drink fluids which were consumed by 301(99.7%), 240(79.5%) and 212(70.2%) participants currently, while current tea consumption was lower than during weaning at 26.2% of 4 year olds. Less than 1% of children consumed herbal tea, sugarless-ready to drink or liquid fruit concentrate either during weaning or currently. More than 70% of the participants consumed cooked yam/cassava/maize products, soup, cooked rice and beans dishes during weaning and also currently. Less than 3% consumed cooked meat, cereals and roasted yam/cassava/maize products during weaning and less than 3% currently consumed confectioneries and cereals.

5.4.3.8 Tooth cleaning practices

The majority 299 (99.0%) of parents or legal guardians of 4 year old study participants reported using toothpaste to clean their child/ward's teeth while 3 (1.0%) reported using salt. In terms of frequency, 253 (83.8%) reported that they cleaned participant's teeth once daily and 269 (89.1%) rinsed after toothpaste use (Table 5.14).

Table 5.15 presents the age at which 4 year old study participants started to clean their teeth. The majority 170(56.3%) of parents or legal guardians reported that they started cleaning participants teeth between age 12 and 19 months and 8(2.6%) started at ≤ 6 months.

The mean (SD) weight of toothpaste dispensed and used was 0.54(0.27) g. The majority 154(51.5%) of the four year olds who used toothpaste used about 0.5 to 0.75 gram while 19.4% reported using 0.88-1.0 g (Table 5.16).

Food and drink groups	During weaning[1]		Currently consumed[1]	
	No. of children	(%)	No. of children	(%)
Drink group				
Water	300	99.3	301	99.7
Liquid milk or yoghurt	128	42.4	131	43.4
Tea	119	39.4	79	26.2
Sugared Ready to Drink	86	28.5	212	70.2
Powdered milk	79	26.2	1	0.3
Carbonated drink	75	24.8	240	79.5
Coffee or chocolate	25	8.3	21	7.0
Powdered fruit concentrate prepared at home	11	3.6	11	3.6
Liquid fruit concentrate prepared at home	6	2.0	10	3.3
Herbal tea	2	0.7	0	0
Sugarless Ready to Drink	1	0.3	1	0.3
Liquid fruit concentrate purchased	0	0	0	0
Food group				
Cooked yam or cassava or maize products	287	95.0	285	94.4
Soup	244	80.8	293	97.0
Cooked rice and beans dishes	226	74.8	293	97.0
Cooked vegetables dishes	191	63.2	287	95.0
Pasta/noodles	103	34.1	204	67.5
Bread	58	19.2	134	44.0
Cooked fish	18	6.0	36	11.9
Fruits	12	4.0	36	11.9
Confectioneries	9	3.0	8	2.6
Cooked meat	5	1.7	31	10.3
Cereals	4	1.3	3	1.0
Roasted yam or cassava or maize products	2	0.7	0	0
Roasted meat	0	0	2	0.7
Roasted fish	0	0	0	0

Table 5-13: Parents or legal guardians' self-reported weaning and currently consumed drinks and foods consumed by 4 year old study participants (n=302). *Note: [1] - Multiple responses*

Frequency of cleaning teeth	No.	(%)
Once daily	253	83.8
Twice daily	46	15.2
>twice daily	3	1.0
Total	**302**	**100.0**
Post teeth cleaning behaviour	**No.**	**(%)**
Rinse after toothpaste use	269	89.1
Spit out after toothpaste use	24	7.9
Does not rinse or spit out after toothpaste use	6	2.0
Rinse after cleaning teeth with salt	3	1.0
Total	**302**	**100.0**

Table 5-14: Oral hygiene habits for 4 year old participants (n=299).

Age of starting to clean teeth	No.	(%)
≤6 months	8	2.6
7-11 months	28	9.3
12-18 months	170	56.3
>18 months	96	31.8
Total	**302**	**100.0**

Table 5-15: Age at which 4 year old study participants started to clean their teeth (n=302).

Amount of toothpaste (g)	No.	(%)
0.06 – 0.25	87	29.1
0.5 – 0.75	154	51.5
0.88 – 1.0	58	19.4
Total	**299**	**100.0**

Table 5-16: Amount of toothpaste used by 4 year old participants who used toothpaste (n = 299).

5.4.3.9 Relationship between prevalence and severity of dental fluorosis in primary teeth and F exposure from drinking and cooking water

Based on the TF index, Figure 5.1 shows that the prevalence of dental fluorosis in primary teeth was 5.9%, 4.3% and 6.3% among participants who drank low, moderate and high F concentration drinking water respectively. Based on the F concentration of cooking water used by each 4 year old, the prevalence of fluorosis was 5.5%, 8.0% and 6.7% for consumption of diets cooked with low, moderate and high F concentration water respectively. When the relationship between the severity of dental fluorosis measured by mean severity score and the F concentration of cooking water or drinking water used by the four year old participants was explored, Table 5.17 shows that there was a weak relationship between the prevalence and severity of dental fluorosis in primary teeth and F exposure in drinking water among 4 year olds (ρ=0.12; p=0.04) while there was no relationship with cooking water (ρ=0.09; p=0.12). The correlations coefficient was 0.17 for drinking water at p=0.007 for water < 0.7 ppmF.

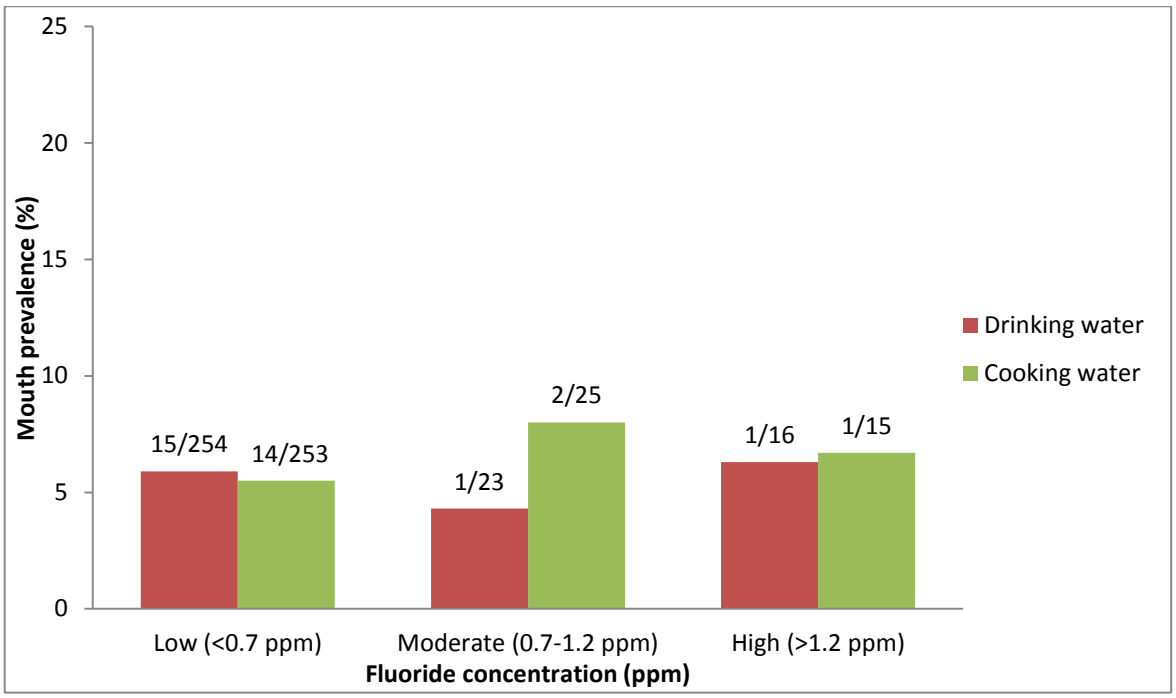

Figure 5-1: Fluoride concentration in drinking and cooking water and presence of dental fluorosis in the primary dentition of 4 year olds (n=302[1]). *Notes: [1] Of the 302 four year old children dentally examined, 293 provided drinking and cooking water samples; Figures above chart represent actual numbers of children with dental fluorosis*

Water (ppm F)	n	ρ	R^2	y	p
<0.7					
Drinking	254	0.169	0.005	0.024+0.08x	0.01
Cooking	253	0.107	4.779^{-4}	0.02+0.03x	0.09
0.7 – 1.2					
Drinking	23	- 0.125	0.002	$0.01-9.09^{-3}$x	0.57
Cooking	25	- 0.066	0.008	0.03-0.02x	0.76
>1.2					
Drinking	16	- 0.174	0.030	0.07-0.02x	0.52
Cooking	15	- 0.161	0.026	0.07-0.02x	0.566
All areas					
Drinking	293	0.115	2.481^{-5}	0.02-1.23x	0.04
Cooking	293	0.092	1.094^{-4}	$0.02-2.74^{-3}$x	0.12

Table 5-17: Correlation between F concentration in drinking and cooking water and the severity of dental fluorosis among 4 year old participants (n=302[1]). *Notes: ρ = Spearman correlation coefficient, correlation equation, y = a+b(x) where y is severity, a is the intercept and b is the slope; [1] Of the 302 four year olds dentally examined, 293 provided drinking and cooking water samples*

5.4.4 Dental Health and Nutrition - Eight year olds

5.4.4.1 Mean (SD) and Median (minimum, maximum) of fluoride concentration (mgF/L) in drinking and cooking water samples

Table 5.18 shows that mean (SD) of fluoride concentration (mgF/L) in drinking and cooking water ranged from 0.24 (0.14) to 1.16 (1.02) and this difference were statistically significant ($p<0.001$). The difference in the mean (SD) of fluoride concentration (mgF/L) in drinking and cooking water in the two rural areas (Area 2 and 4) was statistically significant different ($p<0.05$) but was not in the two urban areas of Areas 1 and 3 ($p>0.05$). The F concentration of drinking water sample of 63 (19.6%) eight year-olds had the same F concentration as their cooking water sample.

5.4.4.2 Socio-demographic data

The mean (SD) age ranged from 8.49(0.31) in Area 1 to 8.58(0.29) in Area 2 and the difference was not statistically significant ($p=0.11$) (Appendix AH). The distribution of males ranged from 48.1% in Area 1 to 59.0% in Area 4 while the distribution of females ranged from 41.0% in Area 4 to 65.8% in Area 2.The distribution of males and females across the 4 Areas was statistically significantly different ($p=0.02$) (Appendix AH).

The duration of residence in present location was not statistically significantly different among males across the areas ($p=0.35$) but was statistically significantly different among females across the areas ($p<0.001$) (Appendix AI). The majority 233(72.4%) had lived in their respective areas from birth while 89(27.6%) had lived in the areas for a shorter period. In the rural Areas 2 and 3 the majority (89.8% and 72.3% respectively) while in urban Areas 1 and 3, (56.8% and 59.2% respectively) had lived in these areas since birth ($p<0.001$).

Appendix AJ shows that parents or legal guardians who never went to school ranged from 16.7% in Area 2 to 28.9% in Area 4. The table also shows that 13(4.0%) were unemployed or were housewives while 309(96.0%) engaged in one form of occupation. The majority 191(59.3%) reported that they earned between N5, 000 – N10, 000 while 17(5.2%) reported that they earned above N40, 000.

Water samples	**Area 1 (Urban, Higher F) (n=81)**	**Area 2 (Rural, Higher F) (n=79)**	**Area 3 (Urban, Lower F) (n=79)**	**Area 4 (Rural, Lower F) (n=83)**	**All Areas (n=322)**	**p value***	**Tukey Post-hoc**
Drinking water samples							
No. where F <0.1 mgF/l[a]	64	20	62	50	196	<0.001	1v2(p=0.001)
No. where F not measured[b]	2	1	0	0	3		2v4(p<0.001)
No. where F ≥0.1	15	57	17	34	123		
Mean (SD) F conc. (mgF/l)	0.25 (0.20)	1.11 (1.00)	0.75 (0.76)	0.27 (0.14)	0.72 (0.84)		
Median (Min, Max)(mgF/l)	0.20 (0.10, 0.80)	0.60 (0.20, 4.00)	0.40 (0.10, 2.00)	0.20 (0.10, 0.50)	0.40 (0.10, 4.00)		
Cooking water samples							
No. where F <0.1 mgF/l[a]	52	24	54	53	183	<0.001	1v2(p<0.001)
No. where F not measured[b]	2	1	0	0	3		2v3(p=0.002)
No. where F ≥0.1	27	53	25	31	136		2v4(p<0.001)
Mean (SD) F conc. (mgF/l)	0.24 (0.14)	1.16 (1.02)	0.56 (0.45)	0.27 (0.13)	0.67 (0.78)		
Median (Min, Max)(mgF/l)	0.20 (0.1, 1.00)	0.90 (0.2, 4.00)	0.40 (0.1, 2.00)	0.20 (0.10, 0.50)	0.40 (0.10, 4.00)		

Table 5-18: Mean (SD) and median (minimum, maximum) of fluoride concentration (mg/L) in drinking and cooking water samples for 322 8-year old participants by area. Notes: [a] - *Low Fluoride concentration (<0.1ppmF) below the detection limit of Fluoride electrode*; [b] - *Not measured = Sample not collected;* [*] - *One way ANOVA*

5.4.4.3 Prevalence and severity of Developmental Defects of Enamel (DDE) in Primary teeth of 8 year-olds

Mouth prevalence

Table 5.19 summarises the key dental health characteristics of the primary dentition of 8 year old participants, while more detailed results are provided in the Appendices.

As Appendix AK shows that the prevalence of DDE in primary teeth of 8 year-old male participants varied between areas from 17.7% in Area 2 to 44.3% in Area 3 ($p<0.001$) while among female participants it was 18.1% in Area 4 to 43.0% in Area 2 ($p=0.06$). There was no statistically significant difference between males and females ($p=0.25$). Table 5.19 shows that mouth prevalence of DDE ranged from 42.2% in Area 4 to 81.0% in Area 3 ($p<0.001$). The mouth prevalence of participants with diffuse opacities ranged from 10.8% in Area 4 to 72.8% in Area 1 (Appendix AL). Overall, the mouth prevalence of diffuse opacities (38.5%) was highest followed by other types of defects (24.2%).

Tooth prevalence

Table 5.19 shows that overall, the mean (SD) number of teeth affected ranged from 0.94(1.28) in Area 4 to 2.88(2.27) in Area 1 ($p<0.001$). Tukey Post-hoc test shows that the mean(SD) number of teeth affected was not statistically significantly different only between Areas 1 and 3, and Areas 2 and 3 ($p>0.05$).Appendix AM presents the mean(SD) and median(minimum, maximum) number of primary teeth affected by DDE by area and gender. There was a statistically significant difference in the mean (SD) number of primary teeth affected by DDE among males and females across areas ($p<0.05$).

A total of 1467 primary index teeth were dentally examined, of which 633(43.1%) had enamel defects while 814(55.5%) had enamel defects (Appendix AN). Defects on 20(1.4%) teeth could not be recorded because buccal surfaces were not available for examination due to accumulation of debris and dental caries. Of the 633 index teeth with defects, 53.2% were diffuse opacities (Appendix AN).

Table 5.20 shows that the upper right 167(26.4%) and left 164(25.9%) first primary molars are the teeth mostly affected while upper right central incisor and lower left and right left second primary molars were the teeth least affected 3(0.5%).

DDE, Dental fluorosis and Dental caries	Area 1 (Urban, Higher F) n=81	Area 2 (Rural, Higher F) n = 79	Area 3 (Urban, Lower F) n = 79	Area 4 (Rural, Lower F) n = 83	All areas n = 322	P values	Post-hoc tests
DDE							
Mouth prevalence (DDE > 0) No. (%)	62 (76.5)	48 (60.8)	64 (81.0)	35 (42.2)	209 (64.9)	< 0.001^{+}	
Tooth prevalence (No. of teeth affected) Mean (SD)	2.88 (2.27)	2.01 (1.87)	2.32 (1.53)	0.94 (1.28)	2.03 (1.91)	<0.001$^{\#}$	1v2*; 2v4**; 1v4**; 3v4**
Dental fluorosis							
Mouth prevalence (Direct clinical examination TFI > 0) No. (%)	10 (12.3)	17 (21.5)	0 (0)	3 (3.6)	30 (9.3)	<0.001^{+}	
Tooth prevalence (No. of teeth affected) Mean (SD)	0.64 (1.97)	1.09 (2.51)	0 (0)	0.08 (0.47)	0.45 (1.66)	<0.001$^{\#}$	2v3**; 2v4**
Dental caries							
Mouth prevalence (dmft > 0) No. (%)	29 (35.8)	7 (8.9)	16 (20.3)	2 (2.4)	54 (16.8)	<0.001^{+}	
Tooth prevalence (No. of teeth affected) Mean (%)	0.95 (1.69)	0.15 (0.60)	0.53 (1.29)	0.07 (0.49)	0.43 (1.18)	<0.001$^{\#}$	1v2**; 1v4**

Table 5-19: Summary of prevalence of DDE, Dental Fluorosis and Dental Caries in primary teeth of 8 year-old participants by area.

*Notes: $^{+}$ - Chi-square; $^{\#}$ - One way ANOVA; * - p<0.01; ** - P<0.001*

Primary teeth	No.	%
Upper right first molar	167	26.4
Upper left first molar	164	25.9
Upper right canine	136	21.5
Upper left canine	111	17.5
Upper right lateral incisor	24	3.8
Upper left lateral incisor	18	2.8
Upper right central incisor	3	0.5
Upper left central incisor	4	0.6
Lower left second molar	3	0.5
Lower right second molar	3	0.5
Total	**633**	**100.0**

Table 5-20: Frequency distribution of the 633 indexed primary teeth with Developmental Defects of Enamel among 8 year-old participants.

5.4.4.4 Prevalence and severity of Dental Fluorosis in Primary teeth of 8 year-olds

Mouth prevalence of Dental Fluorosis

As Table 5.19 describes, the prevalence of dental fluorosis ranged between 0% in Area 3 to 21.5% in Area 2 and these differences in the overall mouth prevalence of dental fluorosis among participants across the areas were statistically significant ($p<0.001$). Appendix AO shows no statistically significant difference in the mouth prevalence of dental fluorosis between males and females ($p=1.00$).

Tooth prevalence of Dental Fluorosis

Table 5.19 shows that no participant's teeth in Area 3 was affected by fluorosis while 1.09(2.51) teeth were fluorosed among participants in Area 2 ($p<0.001$). The difference in mean (SD) number of primary teeth affected by dental fluorosis was statistically significant between Areas 2 and 3 and between Areas 2 and 4 ($p<0.05$). Appendix AP shows no statistically significant difference in the mean (SD) number of teeth affected by dental fluorosis between males and females ($p=0.83$).

5.4.4.5 Prevalence and severity of Dental Caries in Primary teeth of 8 year-olds

Mouth prevalence of Dental Caries

In Table 5.19, 29(35.8%) and 16(20.3%) in the Urban Areas 1 and 3 respectively had dental caries while 7(8.9%) and 2(2.4%) in the rural Areas 2 and 4 respectively had dental caries ($p<0.001$). There was a no statistically significant difference in the mouth prevalence of dental caries between males and females ($p=1.00$) (Appendix AQ).

Tooth prevalence of Dental Caries

Appendix AR shows that the mean (SD) number of primary teeth affected by dental caries ranged from 0.08(0.57) in Area 4 to 0.87(1.49) in Area 1 among males (p=0.003) while it ranged from 0.06(0.36) in Area 4 to 1.02(1.87) in Area 1 among females (p=0.001). There was no statistically significant difference in the mean (SD) number of primary teeth affected by dental caries between males and females (p=0.51). Table 5.19 shows that overall, the mean (SD) number of primary teeth affected by dental caries ranged from 0.07(0.49) in Area 4 to 0.95(1.69) in Area 1 (p<0.001).

5.4.4.6 Relationship between prevalence and severity of Dental Fluorosis in primary teeth of 8 year-olds and Fluoride exposure from drinking and cooking water

The prevalence of dental fluorosis in primary teeth among8 year-old participants who drank low, moderate and high F concentration water was 8.5%, 7.1% and 22.7% respectively while among those whose diets were cooked with low, moderate and high F concentration water it was 8.5%, 10.0% and 22.2% respectively (Figure 5.2). Table 5.21 shows that the correlation between fluoride concentration in drinking water and the severity of dental fluorosis was 0.15 (p=0.01) while it was 0.08 (p=17) when fluoride concentration in cooking water and the severity of dental fluorosis were also correlated. The correlations coefficient was 0.13 for drinking water at p=0.04 for water < 0.7 ppmF.

5.4.4.7 Prevalence and severity of Developmental Defects of Enamel (DDE) in Permanent teeth of 8 year-olds

Mouth prevalence

The prevalence of DDE in permanent teeth of 8 year-old male participants varied between areas from 26.6% in Area 2 to 39.5% in Area 1 (p=0.03) while among female participants it was 19.3% in Area 4 to 58.2% in Area 2 (p<0.001) (Appendix AS). There was no statistically significant difference between males and females (p=0.64). Table 5.22 shows that overall the prevalence of DDE in permanent teeth it ranged from 51.8% in Area 4 to 84.8% in Area 2 (p<0.001).

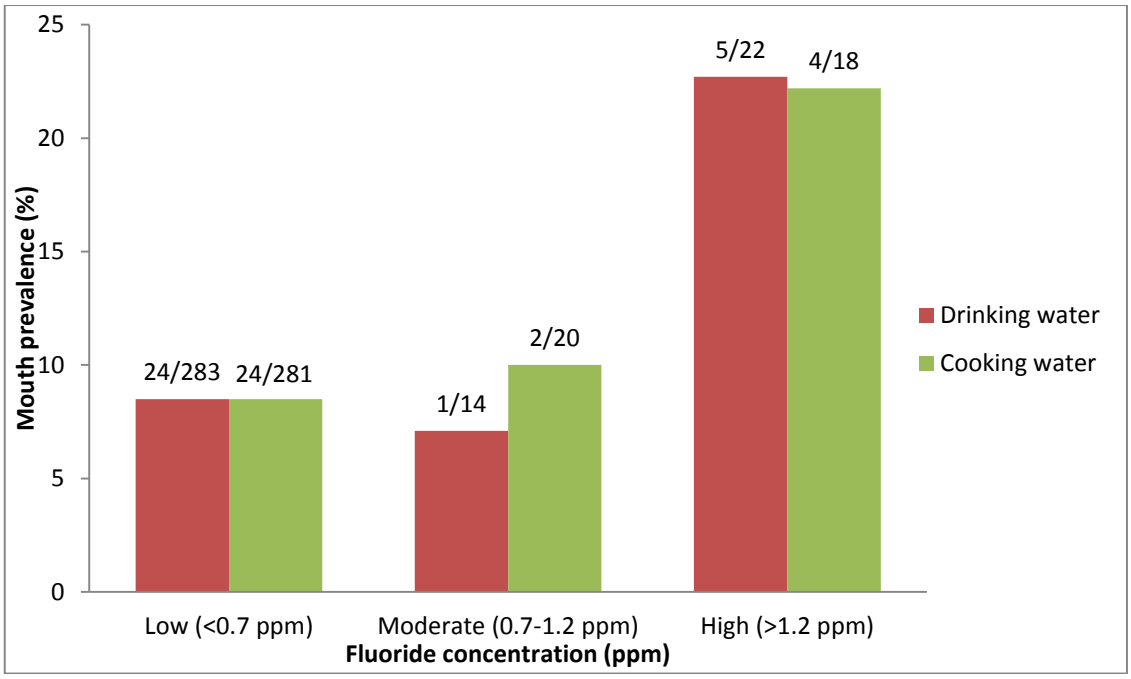

Figure 5-1: Fluoride concentration in cooking and drinking water and presence of dental fluorosis in the primary dentition of 8 year old participants (322[2]). *Notes: [2] Of the 322 eight year old children dentally examined, 319 provided drinking and cooking water samples; Figures above bar charts represent actual numbers of children with dental fluorosis*

Water (ppm F)	n	ρ	R^2	y	p
<0.7					
Drinking	283	0.125	0.02	0.4+0.27x	0.04
Cooking	281	0.042	0.007	0.06+0.19x	0.487
0.7 – 1.2					
Drinking	14	0.324	0.096	- 0.89+1.1x	0.26
Cooking	20	- 0.205	40.227	2.1-2.1x	0.39
>1.2					
Drinking	22	0.092	0.030	- 0.3+0.25x	0.68
Cooking	18	- 0.136	0.041	0.38-0.1x	0.59
All areas					
Drinking	319	0.148	0.036	0.05+0.11x	0.01
Cooking	319	0.079	0.002	0.08+0.02x	0.16

Table 5-21: Correlation between F concentration in drinking and cooking water and the severity of dental fluorosis in primary teeth of 8 year old participants (n=322[2]).

Notes: ρ = Spearman correlation coefficient, correlation equation, y = a+b(x) where y is severity, a is the intercept and b is the slope; [2] Of the 322 eight year olds dentally examined, 319 provided drinking and cooking water samples.

Tooth prevalence

Table 5.22 shows that overall, the mean (SD) number of permanent teeth affected by DDE ranged from 1.24(1.61) in Area 4 to 3.85 (1.95) in Area 2 ($p<0.001$). A Tukey Post-hoc test showed that the mean(SD) number of teeth affected differed significantly between Areas 1 v 2; 2 v 3; 2 v 4 and 1 v 4 ($p<0.01$). Appendix AT describes the statistically significant difference in the mean (SD) number of permanent teeth affected by DDE among males and females across areas ($p<0.001$). The difference in the number of permanent teeth affected by DDE between males and females was not statistically significant ($p=0.27$).

A total of 1655 permanent index teeth were dentally examined for DDE, of which 706(42.7%) had enamel defects while 913(55.2%) had no enamel defects (Appendix AU). Defects on 36(2.2%) teeth could not be recorded because buccal surfaces were not available for examination due to accumulation of debris and dental caries. Of the 706 index teeth with defects, 461(65.3%) were diffuse opacities (Appendix AU).

Table 5.23 shows that upper right (126 (17.8%)) and left (125 (17.7%)) central incisors were the teeth mostly affected by DDE while upper right first premolars (2 (0.3%)) and right canines (3(0.4%)) were the teeth least affected.

5.4.4.8 Prevalence and severity of Dental Fluorosis in Permanent teeth of 8 year olds

Mouth prevalence of Dental Fluorosis

As the summary table (Table 5.22) describes, when measured using all 3 indices; Deans, TFI and photographic imaging with TFI, dental fluorosis was least prevalent (5.1%, 5.1% and 13.9% respectively) in Area 3 (Urban, Lower F) and most prevalent (82.3%, 82.3% and 60.8% respectively) in Area 2 (Rural, Higher F) and the differences between areas were statistically significant for all 3 indices used ($p<0.001$). As Appendices AV and AW and AX show, the differences in mouth prevalence of dental fluorosis among male and female participants across the areas were statistically significant when Deans Index, the TFI index and photographic imaging with the TFI index were used (all $p<0.001$).

DDE, Dental fluorosis and Dental caries	Area 1 (Urban, Higher F) n=81	Area 2 (Rural, Higher F) n = 79	Area 3 (Urban, Lower F) n = 79	Area 4 (Rural, Lower F) n = 83	All areas n = 322	P values	Post Hoc Test
DDE							
Mouth prevalence (DDE > 0) No. (%)	59 (72.8)	67 (84.8)	47 (59.5)	43 (51.8)	216 (67.1)	< 0.001^{+}	
Tooth prevalence (No. of teeth affected) Mean (SD)	2.48 (1.99)	3.85 (2.17)	1.95 (2.25)	1.24 (1.61)	2.37 (2.22)	<0.001$^{\#}$	1v2**; 2v3**; 2v4**; 1v4**
Dental fluorosis							
Mouth prevalence (Deans index) No. (%)	20 (24.7)	65 (82.3)	4 (5.1)	5 (6.0)	94 (29.2)	<0.001^{+}	
Mouth prevalence (Direct clinical examination TFI > 0) No. (%)	20 (24.7)	65 (82.3)	4 (5.1)	7 (8.4)	96 (29.8)	<0.001^{+}	
Mouth prevalence (Digital photographic TFI > 0) No. (%)	30 (37.0)	48 (60.8)	11 (13.9)	14 (16.9)	103 (32.0)	<0.001^{+}	
Tooth prevalence (No. of teeth affected) Mean (SD)	1.22 (2.58)	6.37 (4.02)	0.23 (1.33)	0.64 (2.27)	2.09 (3.66)	<0.001$^{\#}$	1v2**; 2v3**; 2v4**
Dental caries							
Mouth prevalence (DMFT > 0) No. (%)	11 (13.6)	3 (3.8)	10 (12.7)	0 (0)	24 (7.5)	<0.001^{+}	
Tooth prevalence (No. of teeth affected) Mean (%)	0.26 (0.70)	0.06 (0.33)	0.22 (0.67)	0 (0)	0.13 (0.52)	<0.001$^{\#}$	1v4(p=0.01) 3v4(p=0.04)

Table 5-22: Summary of prevalence of DDE, Dental Fluorosis and Dental Caries in permanent teeth of 8 year-old participants by area.

Notes: $^{+}$ - Chi-square; $^{\#}$ - One way ANOVA; ** - P<0.001.

Permanent teeth	No.	%
Upper right first premolar	4	0.6
Upper right canine	3	0.4
Upper right lateral incisor	68	9.6
Upper right central incisor	126	17.8
Upper left central incisor	125	17.7
Upper left lateral incisor	65	9.2
Upper left canine	4	0.6
Upper right first premolar	2	0.3
Lower left first molar	155	22.0
Lower right first molar	154	21.8
Total	**706**	**100.0**

Table 5-23: Frequency distribution of permanent teeth with Developmental Defects of Enamel among 8 year-old participants (n=322).

Tooth prevalence of Dental Fluorosis

Table 5.22 shows that there was a statistically significant difference between Areas in the mean (SD) number of permanent teeth with dental fluorosis ($p<0.001$). The overall mean (SD) number of fluorosed teeth was 2.09(3.66) ranging from 0.23(1.33) in Area 3 to 6.37(4.02) in Area 2. The difference in mean (SD) number of permanent teeth affected by dental fluorosis was statistically significant between Areas 1 and 2; Areas 2 and 3 and Areas 2 and 4 ($p<0.05$). Appendix AY shows a statistically significant difference in the mean (SD) number of teeth affected by dental fluorosis between males and females ($p=0.01$).

5.4.4.9 Prevalence and severity of Dental Caries in Permanent teeth

Mouth prevalence of Dental Caries

In Table 5.22, 24(7.2%) participants had dental caries in permanent teeth with 11(13.6%) and 10(12.7%) in the urban Areas 1 and 3 respectively and 3(3.8%) and none in the rural Areas 2 and 4 respectively ($p<0.001$). Appendix AZ shows a statistically no significant difference in the mouth prevalence of dental caries between males and females ($p=0.84$).

Tooth prevalence of Dental Caries

Appendix BA shows that the mean (SD) number of primary teeth affected by dental caries ranged from 0(0%) in Area 4 to 0.33(0.84) in Area 1 among males ($p=0.02$) while it ranged from 0(0%) in Area 4 to 0.19(0.55) in Area 1 among females ($p=0.21$). There was no statistically significant difference in the mean (SD) number of primary teeth affected by dental caries between males and females ($p=0.78$). Overall, Table 5.22 shows the mean

(SD) number of primary teeth affected by dental caries ranged from 0(0%) in Area 4 to 0.26(0.70) in Area 1 ($p<0.001$).

5.4.4.10 Health during infancy and childhood

Three hundred and seventeen (98.4%) parents or legal guardians reported that the birth of their child or ward was normal while 5 reported that it was an abnormal birth. Whooping cough, measles and diarrhoea were the main illnesses reported during infancy and childhood by 68(21.1%), 49(15.2%) and 48(14.9%) parents or legal guardians respectively while recurrent viral infections was reported by 1 parent/career (Table 5.24).

Type of infancy or childhood illnesses	**No[1].**	**(%)**
Whooping cough	68	21.1
Measles	49	15.2
Diarrhoea	48	14.9
Chicken pox	17	5.3
Pneumonia	15	4.6
Trauma to the face	12	3.7
Rheumatic fever	9	2.8
Trauma to teeth	5	1.6
Recurrent viral infections	1	0.3
Total with illnesses reported	**146**	**45.3**
Total with no illnesses reported	176	54.7
Overall Total	**322**	**100.0**

Table 5-24: Infancy or childhood illnesses among 8 year old study participants (n=322[1]). *Note: [1] - Multiple responses*

Table 5.25 shows that the majority (23(33.8%) and 32(65.3%)) who had reported whooping cough and measles respectively had suffered this at >18 months of age. The majority (14(29.2%)) who had reported diarrhoea had it when they were between 12 and 18 months of age, although the distribution of this illness across the ages when the illness occurred was very even.

Age of having illnesses	**Whooping cough**		**Measles**		**Diarrhoea**	
	No.	**(%)**	**No.**	**(%)**	**No.**	**(%)**
≤6 months	21	30.9	4	8.2	10	20.8
7 – 11 months	5	7.4	3	6.1	13	27.1
12 – 18 months	19	27.9	10	20.4	14	29.2
>18 months	23	33.8	32	65.3	11	22.9
Total	**68**	**100.0**	**49**	**100.0**	**48**	**100.0**

Table 5-25: Age at which 8 year old study participants had whooping cough (n=68), measles (n=49) and diarrhoea (n=48).

Twenty one (6.5%) parents or legal guardians reported that child or wards' family had discoloured teeth; 7(33.3%) and 14(66.7%) of them stated that it affected paternal and maternal family members respectively.

5.4.4.11 Infant and childhood feeding practices

Two hundred and eighty seven (89.1%) parents or legal guardians reported that study participants were exclusively breast fed while 35(10.9%) reported mixed feeding (Table 5.26).

Feeding practices	No.	%
Exclusive breast feeding	287	89.1
Mixed feeding	35	10.9
Exclusive formula feeding	0	0
Total	**322**	**100.0**

Table 5-26: Parents or guardians' self-reported feeding practices of 8 year old study participants (n=322).

The majority of parents or legal guardians reported that child/ward stopped breastfeeding when they were older than 11 months while 1(0.3%) reported that it was stopped before 2 months of age (Table 5.27).

Age	No.	%
< 2 months	1	0.3
2 – 5 months	6	1.9
6 – 11 months	40	12.4
≥ 12 months	275	85.4
Total	**322**	**100.0**

Table 5-27: Age when 8 year old participants stopped breastfeeding.

5.4.4.12 Drinks and foods consumed during weaning and currently

Table 5.28 shows that water, carbonated drink, liquid milk or yoghurt and tea were the drinks mostly consumed during weaning by 320(99.4%), 314(97.5%), 134(41.6%) and 116(36.0%) of participants respectively. The most popular currently consumed drinks were water, carbonated drinks and sugared-ready to drink fluids which were consumed by 321(99.7%), 236(73.3%) and 199(61.8%) participants. Current tea consumption was lower than during weaning at 26.1% for 8 year olds. More than 70% of the participants consumed cooked yam/cassava/maize products, soup, cooked rice, and beans dishes during weaning and more than 90% currently consume them. Less than 3% consumed cooked or roasted fish or meat, confectioneries and during weaning and less than 3% currently consumed confectioneries, cereals and roasted fish.

5.4.4.13 Tooth cleaning practices

The majority 316(98.1%) of parents or guardians reported using toothpaste to clean their child/ward's teeth while 6(1.9%) reported using salt. The majority 282(87.6%) reported that they cleaned participant's teeth once daily and 305(94.7%) rinsed after toothpaste use (Table 5.29).

Food and drink groups	**During weaning[1]**		**Currently consumed[1]**	
	No.	**(%)**	**No.**	**(%)**
Drink group				
Water	320	99.4	321	99.7
Carbonated drink	314	97.5	236	73.3
Liquid milk or yoghurt	134	41.6	113	35.1
Tea	116	36.0	84	26.1
Powdered milk	86	26.7	1	0.3
Sugared Ready to Drink	85	26.4	199	61.8
Coffee or chocolate	23	7.1	21	6.5
Liquid fruit concentrate prepared at home	13	4.0	16	5.0
Powdered fruit concentrate prepared at home	11	3.4	0	0
Sugarless Ready to Drink	1	0.3	6	1.9
Herbal Tea	0	0	0	0
Liquid fruit concentrate purchased	0	0	0	0
Food group				
Cooked yam or cassava products	305	94.7	312	96.9
Soup	263	81.7	310	96.3
Cooked rice and beans dishes	244	75.8	312	96.9
Cooked vegetables dishes	205	63.7	314	97.5
Cooked Pasta/noodles	108	33.5	222	68.9
Bread	52	16.1	139	43.2
Fruits	11	3.4	33	10.2
Cooked fish	9	2.8	31	9.6
Confectioneries	6	1.9	8	2.5
Cereals	6	1.9	3	0.9
Roasted meat	2	0.6	0	0
Cooked meat	1	0.3	34	10.6
Roasted fish	0	0	1	0.3
Roasted yam or cassava products	0	0	0	0

Table 5-28: Parents or legal guardians' self-reported weaning and currently consumed drinks and foods consumed by 4 year old study participants (n=302). *Note: [1] - Multiple responses*.

Frequency of cleaning teeth	No.	(%)
Once daily	282	87.6
Twice daily	36	11.2
>twice daily	4	1.2
Total	**322**	**100.0**
Post tooth cleaning behaviour	**No.**	**(%)**
Rinse after toothpaste use	305	94.7
Spit out after toothpaste use	10	3.1
Does not rinse or spit out after toothpaste use	1	0.3
Rinse after cleaning teeth with salt	6	1.9
Total	**322**	**100.0**

Table 5-29: Oral hygiene habits for 8 year old participants (n=322).

Table 5.30 shows that the majority 162(50.3%) of parents or legal guardians reported that they started cleaning participants teeth between age 12 and 18 months and 16(5.0%) started cleaning participants teeth at ≤ 6 months.

Age of cleaning teeth	No.	(%)
≤6 months	16	5.0
7-11 months	24	7.5
12-18 months	162	50.3
>18 months	120	37.3
Total	**322**	**100.0**

Table 5-30: Age at which 8 year old study participants started to clean their teeth (n=322).

The mean weight of toothpaste dispensed and used was 1.51(0.50) g. The majority 171(54.1%) of the study participants who used toothpaste used about 0.5 to 0.75 gram of toothpaste while 72(22.8%) used 0.06 to 0.25 gram (Table 5.31).

Amount of toothpaste (g)	No.	(%)
0.06 – 0.25	72	22.8
0.5 – 0.75	171	54.1
0.88 – 1.0	73	23.1
Total	**316**	**100.0**

Table 5-31: Amount of toothpaste used by 8 year old participants who used toothpaste (n = 316).

5.4.4.14 Relationship between prevalence and severity of Dental Fluorosis in permanent teeth and Fluoride exposure from drinking and cooking water

Figure 5.3 shows that the prevalence of dental fluorosis in permanent teeth was 24.7% (70/283), 64.3% (9/14) and 59.1% (13/22) among participants who drank low, moderate and high F concentration water respectively while it was 24.5% (69/281), 45.0% (9/20) and 77.8% (14/18) among participants whose diets were cooked with low, moderate and high F concentration water respectively. Overall, for all Areas the correlation between fluoride concentration in drinking water and the severity of dental fluorosis was 0.281 ($p<0.001$)

while it was 0.173 (p=0.002) when fluoride concentration in cooking water and the severity of dental fluorosis were also correlated (Table 5.32). The correlation coefficient was 0.17 (p=0.003) for the 8 year olds drinking water at < 0.7 ppmF.

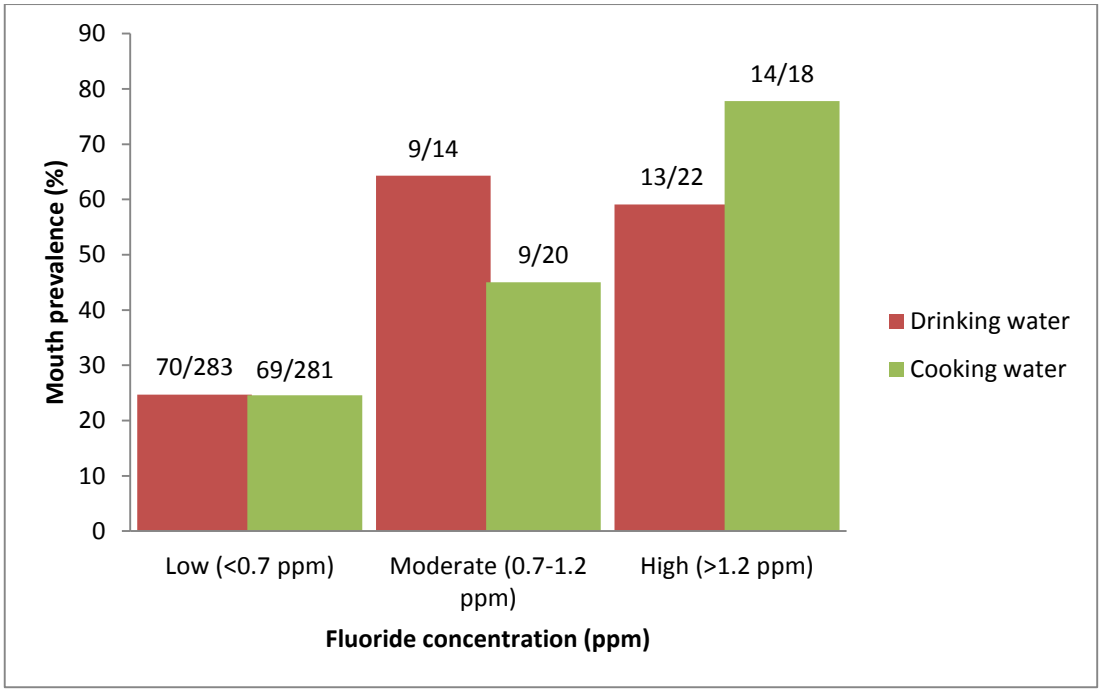

Figure 5-2: Fluoride concentration in cooking and drinking water and presence of dental fluorosis in the permanent dentition of 8 year old participants (322[2]). *Notes: [2] Of the 322 eight year old children dentally examined, 319 provided drinking and cooking water samples; Figures above bar charts represent actual numbers of children with dental fluorosis.*

Water (ppm F)	n	P	R^2	y	P
<0.7					
Drinking	283	0.173	0.014	0.27+0.68x	0.003
Cooking	281	0.021	1.603^{-6}	$0.35-7.25^{-3}x$	0.723
0.7 – 1.2					
Drinking	14	0.593	0.270	- 4.45+5.92x	0.026
Cooking	20	- 0.264	0.187	6.45-6.12x	0.261
>1.2					
Drinking	22	- 0.060	0.003	1.47-0.13x	0.790
Cooking	18	- 0.100	0.006	1.37-0.1x	0.694
All areas					
Drinking	319	0.281	0.064	0.3+0.37x	<0.001
Cooking	319	0.173	0.035	0.33+0.28x	0.002

Table 5-32: Correlation between F concentration in drinking and cooking water and the severity of dental fluorosis in permanent teeth of 8 year old participants (n=322[2]).
Notes: ρ = Spearman correlation coefficient, correlation equation, y = a+b(x) where y is severity, a is the intercept and b is the slope; [2] Of the 322 eight year olds dentally examined, 319 provided drinking and cooking water samples.

5.5 Discussion

5.5.1 Design, study location and sample

This cross-sectional observational survey of 4 and 8 year old children was designed to determine the prevalence and severity of developmental enamel defects and dental caries in a sample of children representative of these age groups in Nigeria. The study was also designed to provide information on F concentration in drinking and cooking water, health during infancy and childhood and infant and childhood feeding and tooth cleaning practices since studies in other countries have shown the influence of these environmental factors on the occurrence of developmental enamel defects and dental caries. The study design was manageable within the available resources and yielded useful results. The study participants were chosen because the findings would be practically beneficial to people in Nigeria and increase local, national and global knowledge on these relevant public health problems in primary and permanent teeth. Naturally fluoridated (higher and lower) water areas in both urban and rural areas were selected to explore the influence of F concentration in water and the presence of enamel defects. In addition, the influence of F concentration in water in these areas on F intake and excretion was also explored since drinking and cooking waters including waters added to other drinks and foods are recognised as one of the main sources of dietary F (Mascarenhas, 2000, Buzalaf et al., 2004). The type and pattern of the defects seen among children in urban and rural communities provided information that will be relevant in the prevention and management of these defects. The study was possible because the inhabitants of Ibadan and Ibarapa where the study was conducted were helpful and cooperative and the researcher was quite familiar with these settings.

Ibarapa, a rural community in southwestern Nigeria was chosen as representative of rural communities because a previous study (Ibiyemi and Taiwo, 2011) on anterior tooth discolouration among adolescents in Igboora, Ibarapa reported the presence of developmental enamel defects. The structure and socio-economic status of this community is comparable with most other rural communities in Nigeria. For example, the adult literacy rate of 62.5% was quite similar to the national adult literacy rate of 65.5% for rural communities (NDHS, 2008). In addition, the primary school enrolment rate of 59% was slightly higher than the national primary school enrolment rate of 57% (NDHS, 2008). Based on the results of the pilot study, Area 2 (ward 1 and 2) and Area 4 (wards 5) were selected as higher and lower water F areas respectively in this rural community. Ibadan, an urban community also in southwestern Nigeria whose socio-economic status and structure

were comparable with other urban communities in Nigeria was selected as representative of urban communities. Area 1 (wards 12 and 6) and Area 3 (ward 3) of Ibadan North LGA were selected as higher and lower water F areas respectively. In Nigeria, the educational system begins from nursery school and ends in university with both public and private institutions. Children within the age of 3 to 5 years attend nursery schools while those between aged 6 to 12 years attend primary schools. Public and private nursery and primary schools in Ibarapa and Ibadan were selected as sampling units for this study because many children attend them though it is not compulsory for parents/legal guardians to send their children/wards to these institutions. In Nigeria, the proportion of children not attending school has been reported as 2.3% in urban areas as against 10.6% for rural areas (NBS, 2010) which might be due to fewer child labour activities in urban compared with rural areas. The study sample was selected by cluster sampling of children in nurseries and primary schools who were aged 4 and 8 years respectively as stated in their school register. Participants within the same cluster were independent of each other since the study design is a cross-sectional observational study. Sampling these children in groups reduced travel and administrative costs and it also made it convenient to recruit them. The sampling was undertaken based on United Nation Educational Scientific and Cultural Organization (UNESCO), 2010 data of 8:1 school enrolment ratio in Nigerian public and private schools. As Figure 4.2 described, based on the sample size calculation resulted in a planned ratio of 7.6:1. When rounded, this ratio was achieved in most areas except for 4 year olds in Area 3 where the ratio was 9.9:1. These were minor differences in sampling ratios and not considered further since a socio-economic analysis was not undertaken as part of the study. The children or wards in the schools used for cluster sampling and whose parents or legal guardians consented to their participation were randomly recruited but data was collected from them based on first come first recruit basis rather than alphabetical order or age order until the complete sample size was achieved. This may have potentially led to a small selection bias in that the keener families might have been first to return their expressions of interest, however since the vast majority (>99%) of the children invited to take part in each cluster did actually participate and the original sampling frame was representative, this bias was minimised. A sample size of 616 children comprising 308 4-year and 308 8-year olds was estimated after making provision for drop out and non-compliance however 624 children comprising 302 4-year and 322 8-year olds participated in the study. The number of the 4 year olds who participated was very slightly lower than expected (by n=6) because a small number of children refused to participate (n= 4) and some parents (n= 2) later

refused to allow their children to participate despite granting written informed consent. The number of 4 year olds who did not participate was very small and should not constitute a significant selection bias since the vast majority of invited individuals did take part. Conversely, the number of 8 year olds who participated was slightly more than expected (by n=14) because those who first attended, motivated their peers who then encouraged their parents/legal guardians to allow them to participate. The fourteen additional 8 year olds may have resulted in a very small selection bias however, the effect of this bias would have been very small because these children represented only 4% of the sample of 8 year olds. The mean ages of the 4 year and 8 year old study participants were 4.5 and 8.5 years respectively, a slight difference in the mean age of 4 year olds in Area 1 and 4 was observed but these were unlikely to be clinically significant in terms of the data being collected. Among the 4 year old participants more males than females participated in the study while more 8 year old females than males participated although these differences were not statistically significant. The male female admission ratio into nurseries and primary schools could be the reason for the observed gender differences. Overall, approximately 70% of study participants had lived in the same study location from birth, although participants who lived in urban settings showed significantly less residency from birth than those who lived in rural settings ($p<0.001$). This implies that participants in the rural higher F areas would have had a longer history of exposure to F from water from their place of residency than those who lived in urban higher F areas.

5.5.2 Socio-economic status of parents or legal guardians of parents

In this study more than 65% of parents or legal guardians were educated and this might be the reason for the high participation of their children/ward. Generally those living in urban areas were more educated than those living in rural areas. It was surprising to observe that parents/legal guardians of 4 years olds living Area 1, an urban setting were the least educated as compared to those living in Areas 2 and 4 which were rural settings. About 5% of parents/legal guardians were not employed and had no income, with more of them in Area 1, the urban setting than the rural settings. These unemployed respondents were mainly housewives who rely on their husbands to meet their needs. Although many parents or legal guardians provided information about monthly income, the overall accuracy of this information was doubtful because a minority were unwilling to provide details of money earned and the income information provided might have been exaggerated. This may be the reason why the parents/carers of 17 (5.6%) 4 year-olds and 12 (3.6%) 8 year-olds reported

that they earned no money at the end of the month or refused to mention what they earned monthly. Furthermore, this was the reason why income was not used as an explanatory variable or predictor of enamel defects in the regression analyses in Chapter 8.

5.5.3 Aim of the study

Few studies have reported the prevalence and severity of DDE (Sawyer et al., 1984, Koleoso, 2004, Orenuga and Odukoya, 2010) and dental fluorosis (El-Nadeef and Honkala, 1998, Wongdem et al., 2001, Akosu et al., 2009) among Nigerian children and these studies did not report the F concentration of water in the environment where the studies were undertaken. Reporting F concentration in water and the prevalence and severity of DDE and dental fluorosis is more meaningful in determining the aetiology of these conditions because F concentration in water used for drinking and cooking is a major factor in the aetiology of the conditions. In addition, the teeth of participants in these Nigerian studies were not dried before they were examined for the presence of DDE or dental fluorosis. Non-cleaning and drying of the surface of the teeth might prevent detailed assessment of tooth surface for enamel defects and cleaning/drying of teeth is essential in ensuring valid and reproducible measurements of tooth surfaces. The tooth prevalence of the defects has also not been reported in these other Nigerian studies therefore information on severity of defects was previously unavailable. Furthermore, these studies were undertaken mostly among children older than 10 years therefore findings from permanent teeth were mainly reported. One study (Orenuga and Odukoya, 2010) was undertaken among 4 to 16 year olds but did not report the prevalence and severity of DDE in primary and permanent teeth separately. Separate information on the prevalence and severity of enamel defects in primary and permanent teeth is important in the formulation of oral health policy on the prevention of pre- and post-natal factors responsible for the occurrence of defects in primary and permanent teeth respectively. In addition, the majority of the Nigerian studies did not report the distribution of factors associated with these defects. An understanding of these is important in trying to mitigate them when planning and making public and dental health policy decisions. This present study thereby presented the prevalence and severity of these defects as well as the distribution of factors associated with the occurrence of these defects among 4 and 8 year old Nigerian children. Findings from this study will be useful in guiding policy decisions in the prevention of developmental enamel defects in Nigeria and other sub-Saharan countries with similar oral health challenges.

5.5.4 Validation and reproducibility of the study

The validation and reproducibility of the data was high, over 80% intra-examiner agreement was recorded for the clinical dental examination, independent scoring of the images of the dentition and measurement of F concentration in drinking and cooking water. The validation of the clinical dental examination was carried out using the worse score of mDDE index for both DDE recordings of the 10% of the study participants that were re-examined.

5.5.5 Fluoride concentration in drinking and cooking water

One of the aims of this study was to determine the relationship between dental fluorosis and F exposure from drinking and cooking water since F concentration in drinking and cooking water has been reported as a major aetiological factor in terms of risk of dental fluorosis. Selection of the areas of study was based on a pilot study of the F concentration of ground water in different areas of Ibadan and Ibarapa as described in Chapter 4 since there was no earlier information on the concentration of F in water either used for drinking or cooking in these areas. Samples of water commonly used by study participants for drinking and cooking during infancy and childhood were collected from parents/legal guardians, however information about their actual sources (i.e. whether borehole, well, tap, bottled or sachet) was not obtained. Reports have shown that ground water from deep or shallow wells is the main source of water in Nigeria. The waters from these wells are usually fetched directly using plastic containers or through hand driven pumps and are presented for consumption through the taps, bottles and sachets. Fluoride analysis of drinking and cooking water samples in the present study showed that the F concentration of about 20% of participants' two water samples (i.e. cooking and drinking water) was equivalent indicating that different water sources but with the same F concentration might have been used for drinking and cooking or that exactly the same water source was used for both drinking and cooking in these individuals. Overall, the F concentration of drinking and cooking water samples consumed by participants varied slightly from the F concentration of their community water supply that was tested in the pilot study and used to select the areas into high and low F water areas. Although the pilot study was undertaken to select the urban and rural areas to represent higher and lower water F areas, there were no huge differences between areas in actual F concentration when F analyses of drinking and cooking water samples were carried out. This is because drinking and cooking water samples provided to the researcher might not have been from the community water supply,

but rather they might have been from water sold in sachets. These sachet waters are distributed widely in Nigeria and may therefore have been transported to the 4 different areas resulting in children in these areas being exposed to more similar F concentrations from drinking water in particular. Other sources of variability in the F concentration in the waters might have been due to the water samples being collected from shallow wells since the finding is in agreement with previous report (Zohouri and Rugg-Gunn, 2000a) that recorded variability in the F concentration of water obtained from shallow wells. In addition, F concentrations of waters collected from shallow wells during a rainy season are usually lower than those collected during a dry season (WHO, 1984). The mean F concentration of drinking water consumed by 4 and 8 year olds were 0.76 ppm and 0.72 ppm respectively and the corresponding figure for cooking water was 0.69 ppm and 0.67 ppm respectively. However in one rural area (Area 2), it ranged between 1.10 ppm and 1.16 ppm which is high when compared to the recommended F concentration for tropical countries like Nigeria (Akpata et al., 2009). As a result, chronic excessive consumption of water during tooth development in Area 2 could increase the risk of development of dental fluorosis and the associated enamel defects.

5.5.6 Choice of indices and methods of measuring DDE, dental fluorosis and caries experience

Different classifications and indices have been used to measure enamel defects for clinical, diagnostic, aetiological and medico-legal purposes. The comparison of the findings of epidemiological surveys of enamel defects in different populations has been complicated by the use of several classifications and indices (Mohamed et al., 2010). The association between enamel defects and several aetiological factors was difficult to establish when studies in different settings were compared due to the different assessment indices employed (Corrêa-Faria et al., 2014). Therefore, there is the need for a consensus agreement on the use of standardised indices so that studies on DDE or fluorosis measurement can be directly compared. In this present study, the appropriate indices listed in WHO Basic Methods for Oral Health Surveys were used. These indices have been used in many other epidemiological surveys. The mDDE index, based on types and appearance of the enamel defects was used to measure and categorise DDE. This index is descriptive and records both F and non-F induced defects, therefore, it allows for the determination of the overall prevalence of enamel defects. The presence of dental fluorosis among study participants was assessed using both the Deans and the Thystrup and Fejerskov indices.

These indices are very popular and widely used in various population surveys of dental fluorosis. The dmft/DMFT indices were used to measure the presence of dental caries in the primary and permanent teeth of the study participants respectively. The indices used to assess developmental enamel defects as well as dental caries are recommended by the World Health organization (WHO) for use in oral health surveys (WHO, 1997).

5.5.7 Methods of measuring developmental enamel defects and dental caries

These indices used in measuring enamel defects and dental caries in this study were used when the teeth of the study participants were measured in the nurseries and primary schools by direct clinical measurement under natural light but not under direct sunshine. When the natural light was not bright enough or when examining the posterior teeth, white light from a dental mirror was used. Prior to the direct dental examination, the participants brushed their teeth and the teeth were dried using a piece of gauze to allow for detail assessment of tooth surfaces. The TF index was also used to macroscopically, indirectly and remotely assess the images of the anterior teeth of the participants for presence of dental fluorosis by an independent examiner. Assessing images of enamel defects remotely has helped in diagnosing and increasing the accuracy in detecting these defects (Cochran et al., 2004b). It also helped to objectively and blindly assess the defects without causing discomfort to the participants and the examiner (Golkari et al., 2011). One reason why direct clinical measurement to complement photographic assessment of tooth surfaces was carried out was because field conditions for taking images can be challenging in tropical conditions due to specular reflections from intense rays of light from broken roofs and windows. However, this challenge was ameliorated by using a black umbrella to block the rays of light. In addition, photographic imaging can be improved if the operator receives specific intense training or if a person is dedicated purely to this task as reported in a previous study (McGrady et al., 2012a).

5.5.8 Prevalence and severity of developmental defects of enamel

Developmental defects of enamel (DDE) are usually classified as either demarcated opacities, diffuse opacities or hypoplasia (FDI, 1992b). Population surveys have shown an increase in the prevalence of DDE across various countries (Seow et al., 2011) and studies (Aine et al., 2000, Contaldo et al., 2014) have reported the presence of aesthetic problems, low self-esteem, dental sensitivity, erosion, wear, dentofacial anomalies and dental caries among people who have DDE. Information on the prevalence and severity of DDE will show the magnitude and extent of the defects in the primary and permanent teeth which will

help inform policy makers on the need to prevent and manage the occurrence of the defects. In this study, the mouth prevalence of DDE in primary dentition was 77.8% and 64.9% among 4 and 8 year olds respectively while in the permanent dentition of 8 year olds the prevalence was 67.1%. The higher mouth prevalence of DDE in primary teeth of 4 year-olds might be due to higher occurrence of prenatal aetiological factors and presence of a complete primary dentition in their mouth when compared to 8 year-olds who are in a mixed dentition. The development of DDE in the permanent teeth is mostly caused by postnatal aetiological factors. The frequency of DDE in primary teeth falls within the range of 3.9%-81.3% reported in primary dentition of 4 and 9 year old American (Wong et al., 2009) and 1 to 4 year old Brazilian (Targino et al., 2011) children respectively. The present prevalence in primary teeth was higher than 3.9%, 10%, 11.2%, 32.6% and 33.3% reported for 5 and 9 year-old American (Hong et al., 2009), 3-5 year-old Brazilian (Lunardelli and Peres, 2005), 4-16 year-old Nigerian (Orenuga and Odukoya, 2010), 1.5-6 year-old Chinese (Lin et al., 2011) and 0.6-3 year-old Tanzanian (Masumo et al., 2013) children respectively. However, it was lower than 79% and 82% reported for 1-3 (Chaves et al., 2007) and 1-4.5 year-old Brazilian (Targino et al., 2011) children. The prevalence of DDE in permanent dentition of 8 year olds in this study is within the range of 9.8% to 92.1% reported for 11-13 year old Italian (Angelillo et al., 1990) and 12 year old Hong Kong (Wong et al., 2006) children respectively. It was higher than 40.2%, 42.5%, 50.1% and 64% reported for 7-13 year-old Brazilian (Soviero et al., 2009), 10 to 19 year old Nigerian children (Koleoso, 2004), 15-16 year-old British (Dummer et al., 1990) and 8-10 year-old Brazilian (Vargas-Ferreira et al., 2014) children respectively but was lower than 83% and 90.7% reported for 14 year-old Saudi Arabia (Rugg-Gunn et al., 1998) and 11-12 year-old Malaysian (Yusoff et al., 2008) children respectively. The differences observed might be due to differences in age group studied and examination conditions. Comparisons of prevalence estimates with other studies should be done with caution since age groups studied and diagnostic criteria used may vary across the studies.

In accordance with some studies, diffuse opacities were the most frequent DDE in both primary (Chaves et al., 2007, Masumo et al., 2013) and permanent (Robles et al., 2013, Vargas-Ferreira et al., 2014) teeth. However, hypoplasia were the most common defects in both primary (Lin et al., 2011) (Santanu et al., 2014) and permanent (Seow et al., 2011) teeth in some studies. In contrast to this present study, in primary (Seow et al., 2011, Corrêa-Faria et al., 2013b) and permanent (Dummer et al., 1990, Mackay and Thomson, 2005) teeth some studies showed that demarcated opacities were the most prevalent defects.

The observed differences in the frequency of types of DDE might be due to different geographical locations and aetiological factors. Evidence shows that among the types of DDE, hypoplasia is the defect mostly associated with early childhood caries (Vargas-Ferreira et al., 2014). Thus, the prevalence of hypoplasia which was 3.4% and 5% among 4 and 8 year olds respectively is not likely to contribute to an increased risk of future caries. The majority of the defects were found in the primary and permanent teeth of participants who lived in high water F areas. Similarly, more teeth of those who lived in high water F areas were affected than those who lived in low water F areas. The higher prevalence and severity of DDE among those who lived in high water F areas might be the reason for the high prevalent of diffuse opacities which could be due to ingestion of F from water. Diffuse opacities of enamel are the feature distinguishing the teeth of children living in low and high fluoridated areas (Cutress et al., 1985). This is particularly so in the permanent teeth where it could occur in permanent teeth of children exposed to optimal ranges of F in drinking water. On the contrary, in the primary dentition of 8 year olds who lived in the urban setting, the majority of the defects were found among those who lived in low F areas. This might probably be due to higher prevalence of non-F aetiological factors in the low F areas than in the high F areas. It might also be due to ingestion of F from non-dietary sources such as toothpaste.

The mean number of primary teeth affected by DDE was higher in 4 year olds than in 8 year olds in accordant with findings reported by (Cruvinel et al., 2012) but it is at variance with higher occurrence of the defects in older age group than in younger age group in previous studies (Li et al., 1995, Masumo et al., 2013). The reason for this difference in prevalence of DDE in primary teeth might be due to differences in race and aetiological factors. The prevalence of DDE was also higher in the permanent teeth of 8 year olds than in their primary teeth and this could be due to presence of more of the former than latter. In accordance with some previous studies (Slayton et al., 2001, Lunardelli and Peres, 2005), in the primary teeth, upper first molars followed by upper canines were teeth mostly affected while a study (Masumo et al., 2013) showed that lower central and lateral incisors were the teeth least affected. On the contrary, some studies (Masumo et al., 2013, Kar et al., 2014) reported that upper central incisors followed by upper canines were teeth most frequently affected while some (Li et al., 1995) reported that lower molars were the teeth least affected. The different observations might be due to the stage of tooth development and stage of tooth eruption at the time of exposure to the various aetiological factors. In this present study only fully erupted primary teeth were examined while in the (Masumo et al.,

2013) study only partially erupted primary teeth were examined. Consistent with previous studies (Soviero et al., 2009), in the permanent teeth, lower first molars followed by upper central incisors were the teeth mostly affected while upper canines and premolars were the teeth least affected. There was no statistically significant difference in the mouth and tooth prevalence of DDE between males and females. This result is in accordant with findings from some studies (Orenuga and Odukoya, 2010, Memarpour et al., 2014) but it is contrary to some studies (Slayton et al., 2001, Masumo et al., 2013) where boys presented with more DDE than girls. A study (Cruvinel et al., 2012) on prevalence of enamel defects among 5 to 10 year olds Brazilian children reported that girls had more defects than boys. Prevalence of DDE might vary between genders because of different geographical locations where studies were undertaken or due to differences in the proportion of males or females in the sample studied.

5.5.9 Prevalence and severity of dental fluorosis

From the available literature, the prevalence and severity of dental fluorosis varies from one study and region to another and according to investigation methods employed (Ng'ang'a and Valderhaug, 1993, Warren et al., 2001, Ruan et al., 2005a). The prevalence of dental fluorosis also varies from place to place depending on the F concentration of the local drinking water. However, in areas with the same F concentration in drinking water, the prevalence and severity may vary greatly due to alternative F sources, dietary habits, climatic conditions and the elevation of the living area (Awadia et al., 1999, Rwenyonyi et al., 2000). Dental fluorosis in primary teeth is considered to be relatively rare (Warren et al., 2001) and/or less severe in comparison to dental fluorosis in the permanent teeth (Thylstrup, 1978, Warren et al., 1999). This has been explained by the placental barrier which prevents transfer of F from mother's blood to the foetus (Warren et al., 1999). Fewer studies have assessed the prevalence and severity of dental fluorosis in sub-Saharan Africa. In this present study, the mouth prevalence of dental fluorosis in primary teeth was 5.6% and 9.3% for 4 and 8 year olds respectively which might be due to age differences since fluorosis increases as age increases due to post-eruptive breakdown of enamel in these teeth as the child gets older. It is this post-eruptive breakdown of enamel that is recorded as a more severe condition. Also, higher prevalence of fluorosis in the primary teeth of 8 year olds might be due to their mixed dentition state where only the primary molars are left which are more likely to have been exposed to systemic F post natally rather than having the placenta barrier to F found with earlier erupting primary teeth. This prevalence falls

within 0% to 100% reported for Swedish children living in less than 0.2ppm (Forsman, 1977) and 10ppm (Forsman, 1974) water F areas respectively. It was lower than 18%, 76.5% and 96.6% reported for 6 to 8 year old Kenya children living in non-fluoridated areas (Ng'ang'a and Valderhaug, 1993), 5-6 year-old Iranian children living in water fluoridated areas (Poureslami et al., 2013) and 7-8 year-old Chinese children living in 7.6 ppm F water areas (Ruan et al., 2005a). The prevalence in primary teeth of 4 year olds was similar to 5.8% reported for 4.5 to 5 year old American children who lived in 0.1ppm water F areas (Warren et al., 2001). The prevalence in primary teeth of 8 year olds was higher than 3.3% and 6.2% reported for 3-5-year-old American children (Leverett et al., 1997) and 7-8 year old Chinese children (Ruan et al., 2005a) living in 0.4 ppm F water areas. The differences in the frequency of distribution of dental fluorosis in the primary teeth might be due to differences in F exposure and investigation methods employed. Dental fluorosis in primary teeth may be overlooked partly because it is less prevalent and less severe when compared to fluorosis in the permanent dentition. In addition primary teeth is often neglected because it is temporary. Conversely, fluorosis in primary teeth should be given the required attention because a previous study (Milsom et al., 1996) on enamel defects in primary teeth reported that children with enamel defects in their primary teeth are also likely to have defects in their permanent teeth. Therefore, the primary teeth may act as a biomarker of F exposure and thus give an indication of what to expect in permanent dentition.

Globally, the prevalence of dental fluorosis in permanent teeth ranges from 4% among 12 year old Lithuanians living in low water F areas (Narbutaite et al., 2007) to 100% among 10 and 15 year old Kenyan children living in areas containing 2ppm water F areas (Manji et al., 1986). The prevalence of 29.8% in the permanent dentition of 8 year olds in this present study falls within this global range. This prevalence was higher than 1%, 4%, and 25% reported for 12-16 year-old Iranian, 12 year-old Lithuanian, 14 year-old British and 8-9 year-old British children living in 0.3ppmF, 0.2 ppmF and less than 0.1 ppmF areas respectively. In Nigerian children it was higher than 12.9% and 26.1% reported for 12-15 (Akosu et al., 2009) and 7-19 (Wongdem et al., 2001) year-olds living in naturally fluoridated areas while it was lower than 51% reported for 12 to 15 year-olds living in less than 0.5 ppm water F areas (El-Nadeef and Honkala, 1998). When compared with other African countries, it was higher than the 7% reported for 12-17 year-old Tanzanian children living in 0.2 ppmF water areas (Mabelya et al., 1997) while it was lower than the 95% and 100% reported for 6-18 year-old Ethiopian (Awadia et al., 2000a) and 12-17 year-old

Tanzanian (Mabelya et al., 1997) children living in 3.6 ppmF and 0.8 ppmF water areas respectively. The very high prevalence of dental fluorosis in the permanent teeth of children in the Ethiopian and Tanzanian studies was reported to be due to non-vegetarian dietary practices and use of "magadi" as a tenderiser among the study participants respectively. Non-vegetarian diets can acidify urine resulting in decreased urinary F clearance and greater F retention which will increase the risk of dental fluorosis; i.e. non-vegetarianism is directly associated with the prevalence of dental fluorosis (Whitford, 1997). Depending on where it is sourced, magadi can contain particularly high levels of F and has been reported to cause severe dental fluorosis in communities with low water F. Differences in F exposure, especially from water, and the age group of participants studied might be the reason for the observed differences in the prevalence of dental fluorosis seen in the permanent teeth of the 8 year old children. A systematic review of 214 studies on water fluoridation showed a significant dose-response association between the F concentration in the drinking water and the prevalence of dental fluorosis(McDonagh et al., 2000).

In this study, the mouth prevalence of dental fluorosis in the primary teeth of 4 year olds was slightly higher when Dean's index was used to assess the defects than when TFI was used which was at variance with result of a previous study (Burger et al., 1987) where the scoring systems produced identical prevalence of fluorosis. The ease with which Dean's index can be employed in epidemiological studies could be the reason why a higher prevalence of dental fluorosis in primary teeth was observed in this present study. Conversely, the two scoring systems produced identical mouth prevalence in the permanent teeth of 8 year olds as reported by (Burger et al., 1987) because the assessment was only based on presence or absence of dental fluorosis. The mouth prevalence of dental fluorosis and the mean number of teeth affected by dental fluorosis was higher in permanent teeth than in primary teeth which was in accordance with results of previous studies (Ng'ang'a and Valderhaug, 1993, Rango et al., 2012, Firempong et al., 2013). The possible reason might be due to time related variations in the F content of drinking water and longer exposure of permanent tooth buds to excessive F ingestion. The prevalence and severity of dental fluorosis was higher in areas of higher water F in both rural and urban areas than their counterparts when the Dean and TF indices were used. These findings were in agreement with previous studies (Cochran et al., 2004a, Meyer-Lueckel et al., 2011, Firempong et al., 2013). The reason for the higher prevalence of fluorosis in higher water F areas might be due to higher ingestion of F water used for drinking and cooking. Further

research is needed to better characterize the link between total F intake and dental fluorosis in both the primary and permanent dentitions.

When TFI was used to assess dental fluorosis indirectly on images of the upper permanent upper central incisors, the mouth prevalence of dental fluorosis was higher than when it was used to assess them directly and clinically. This is in agreement with the report by (Cochran et al., 2004c) which mentioned that photographic methods increase accuracy in detecting enamel defects than clinical examinations. The reason might probably be due to field challenges since image scoring remotely offer participant and examiner comfort. However, the mouth prevalence of dental fluorosis on the primary upper central incisors of 4 year olds when TFI index was used to assess images of these teeth was lower than when all the teeth in their mouth were clinically assessed. This might be due to assessment of more primary teeth by clinical methods than photographic methods. Consistent with other studies (Ng'ang'a and Valderhaug, 1993, Ruan et al., 2005b), there was no statistically significant difference in the prevalence of dental fluorosis in the primary dentition between males and females. However, it was otherwise in the permanent dentition with more females having dental fluorosis than males in accordance with other studies (Wondwossen et al., 2006, Zerihun et al., 2006) which could be due to influence of genetics on the occurrence of dental fluorosis or differences in dietary intake. Two previous studies (Huang et al., 2008, Ba et al., 2011) have opined that the predisposition of individuals living in a community to dental fluorosis is genetically determined. High dietary concentrations of certain cations especially calcium can reduce the extent of F absorption which can reduce the risk to dental fluorosis (Whitford, 1994a).

5.5.10 Prevalence of dental caries

Caries aetiology is multifactorial and a substantial body of knowledge has underlined the role of both socio-demographic and biological influences (Peres et al., 2009). As an example of the biological influences, bacterial such as streptococcus mutans colonise enamel defects metabolise sugar to cause caries in both primary and permanent dentitions (FDI, 1992b, Ellwood and O'Mullane, 1994, Carvalho et al., 2011, Targino et al., 2011). Reports have shown that enamel affected by defects has a lower mineral content compared to sound enamel which allow additional plaque accumulation and colonization by *Streptococcus mutans* and Lactobacilli thereby facilitating greater caries onset and progress than seen in non-defective enamel (Li et al., 1996, Milgrom et al., 2000, Caufield et al., 2012). In this present study, the dmft/DMFT index, a simple index was used to measure

caries experience because of the inclusion of caries data as a contributory or confounding factor.

In Nigeria, the prevalence and severity of dental caries varies because studies were conducted in different age groups using different methods and in diverse populations. The only national data on the prevalence of dental caries in children in Nigeria was conducted in 1995 and showed prevalence of 30% and 43% in children aged 12 years and 15 years respectively (Adegbembo et al., 1995). The prevalence of dental caries in Nigeria ranges from between 5.2% and 48%: higher in urban than in rural areas, higher in Northern than in Southern Nigeria and higher in primary than in permanent teeth (Folayan et al., 2014b). Greater exposure to cariogenic diets was likely to be the main reason for the observed higher prevalence of dental caries seen in the urban compared with the rural areas. In this present study, the mouth prevalence of dental caries in primary teeth was 10.6% and 16.8% among 4 and 8 year olds respectively. The corresponding values for tooth prevalence was 0.29 and 0.43 respectively. The higher prevalence observed in the primary teeth of 8 year olds may be due to their primary teeth having been present in the mouth longer since caries experience is an age-related condition. The mouth and tooth prevalence of dental caries in permanent teeth of 8 year olds was 7.5% and 0.13 respectively. The mouth prevalence of dental caries in both primary and permanent teeth falls within the range reported for Nigeria (Folayan et al., 2014b). The mouth and tooth prevalence was higher in the primary dentition than in permanent dentition which is in accordance with findings from a previous Nigerian study (Folayan et al., 2014a). Increased consumption of a cariogenic diet and poor oral hygiene practices by younger age group might be the reason for the higher prevalence of dental caries in primary teeth than in permanent teeth. In addition, it is more likely that permanent teeth have not been erupted as long in 8 year olds. This was further confirmed by a recent study (Sofola et al., 2014) on changes in the prevalence of dental caries in Lagos, Nigeria which showed that there was a significant increase in the prevalence and severity of dental caries in primary teeth, a decrease in caries prevalence in the permanent teeth and no change in the severity of caries in permanent teeth over a 3 year follow up period. Consistent with previous studies (Akpata, 2004b, Folayan et al., 2014b), caries prevalence in both primary and permanent teeth was higher in urban than in rural areas of Nigeria. There was no statistically significant difference in the mouth and tooth prevalence of dental caries in both primary and permanent teeth between males and females which is consistent with a previous Nigerian study (Okoye and Ekwueme, 2011). This finding is at variance with the study by Sofola et al. (2014) in Lagos, Nigeria that reported a significant

increase in caries prevalence among females which the authors suggested might be due to differences in dietary and tooth cleaning practices.

5.5.11 Health during infancy and childhood

The health of a child during infancy and childhood if affected by environmental factors can contribute to some dental conditions. The genetic control of enamel and dentine formation can be influenced by environmental changes such as systemic medical illnesses, chemical poisons, radiation and trauma (Seow, 1991, Fraga et al., 2005). Acquired systemic factors that are likely to affect enamel development may be conveniently considered as pre-, peri- and postnatal conditions in relation to the timing of the event (Seow and Salanitri, 2013). Prenatal factors which may contribute to enamel hypoplasia include maternal smoking, vitamin C deficiency during pregnancy and neonatal tetany while postnatal factors include nutritional deficiencies such as deficiency in protein, vitamins and iron (Seow and Salanitri, 2013). Preterm children and those with low birth weight have a higher prevalence of enamel hypoplasia compared to children born full term with normal birth weights (Seow et al., 2011) due to trauma to developing tooth bud during intubation. In addition, children with chronic renal failure, coeliac and liver diseases may present with enamel defects due to malabsorption and mineral deficiencies (Páez et al., 2008, Majorana et al., 2010). Clinical reports have suggested that infections of the urinary tract, otitis and upper respiratory disease are associated with enamel defects (Ford et al., 2009). Viral infections such as chicken pox, rubella, measles, mumps, influenza, cytomegalovirus and bacterial infections such as congenital syphilis acquired from maternal Treponema pallidum have also been associated with enamel defects in both primary and permanent teeth (Fraga et al., 2005). In many infections, the causative microorganisms may infect the ameloblasts directly or alter cellular function indirectly through their metabolic products or high fevers induced in the patient (Seow and Salanitri, 2013). In contrast to these systemic factors which usually affect all developing teeth, local factors such as trauma involve only the teeth in the immediate area of damage. For example, trauma exerted on a neonate's maxillary alveolus from laryngoscopy can cause localized defects on the maxillary incisors ranging from mild enamel opacities to severe enamel hypoplasia to crown dilacerations (Seow et al., 1990). Similarly, local trauma exerted through the thin buccal cortical bone is thought to be the cause of demarcated opacities commonly observed on the labial surfaces of primary canines (Lukacs, 1991).

In this study, more than 96% of the parents/legal guardians reported that participants had a normal birth weight and their delivery was without complications. This was higher than what was expected because previous studies reported 10% to 13.2% low birth weight among Nigerian children (Eregie, 1993, Mutihir, 2006). A retrospective study (Onyeka et al., 2011) of birth weight and caesarean delivery in south-east Nigeria reported that 17.6% of children were delivered by caesarean sections. The high prevalence of children born normally might be due to recall or memory bias and parents or legal guardians not telling the truth. About 45% of parents or legal guardians reported that participants had one form of illness during childhood and this could be a major cause of developmental enamel defects. Several studies (Guergolette et al., 2009, Arrow, 2009, Masumo et al., 2013) have reported that infectious diseases during early childhood period have been associated with the occurrence of enamel defects. Medical conditions such as whooping cough, measles, diarrhoea, chicken pox, pneumonia, rheumatic fever, neonatal tetanus, trauma to the face and teeth were reported. These childhood illnesses have been reported in many studies as a possible aetiology of enamel defects (Ford et al., 2009, Arrow, 2009). Though information on previous history of malaria, an endemic illness in tropical countries, was not sought from parents or legal guardians of study participants in this present study, it was assumed that some of the less specific childhood illnesses reported may have been due to malaria. High fever and systemic upset from malaria could have a direct effect on teeth undergoing development at the time of the illness. Further research to investigate the influence of malaria on the occurrence of enamel defects would be useful since no known study has investigated this relationship. In this present study, the reported prevalence of whooping cough was high and similar in both age groups. It was higher than the prevalence of diarrhoea and pneumonia, this could be due to low rate of vaccination of children against whooping cough. In addition, the lower prevalence of diarrhoea and pneumonia might be due to increase awareness to good hand hygiene practices among children in the study locations as a result of the hand hygiene promotion established in schools by various governmental and non-governmental organizations (Agberemi et al., 2009). It was surprising to observe that the prevalence of recurrent viral infections which present as fever, sore throat and swollen parotid glands was reported as low in both age groups (0.3% for both 4 and 8 year-olds). The observed differences in the prevalence of childhood illnesses could be due to respondent or memory bias since a questionnaire containing just one question was used to obtain information from parents or legal guardians about the presence or absence of some listed illnesses during their child's infancy and childhood. A

form of respondent bias where the respondent was unable to provide accurate answers to questions on health during infancy or childhood was pre-empted but opt-out choice in the form of "Don't know" was provided to minimize or eliminate this bias. The opt-out choice eliminated only respondents who were conscience of their lack of knowledge to answer correctly. In this present study, more than 50% of parents or legal guardians whose child/ward fell ill during childhood reported that the illness occurred after 6 months of age. This age period fall within the critical period of tooth development when the teeth could be affected by enamel defects. Children in the first three years of life are mostly susceptible to all forms of dental fluorosis (Buzalaf and Levy, 2011, Wambu et al., 2014). The occurrence of childhood illness was slightly more among younger children than older children, this might due to recall or memory bias since younger children parents or legal guardians have better memory of infancy. About 6.5% of parents or legal guardians reported a family history of tooth discolouration which may be from developmental enamel defect or inherited dental conditions such as Amelogenesis Imperfecta. The presence of tooth discolouration in these parents or legal guardians may indicate a role of genetics in the occurrence of enamel defects as this was alot lower than their offspring. However, this reported low prevalence of family history of tooth discolouration could be due to inability of parents or legal guardians to identify tooth discolouration or respondent bias and the threshold for describing discolouration as such may be much higher in parents/carers than in dentists who are trained to identify all discolourations/defects using specific indices. In addition a parent/carer may not describe something as discolouration unless it was more severe.

5.5.12 Infant and childhood feeding practices

Adequate nutrition during infancy and childhood is essential not only for overall physical health but also for the development and maintenance of the teeth. The influence of stunting, wasting and malnutrition in the occurrence of enamel defects will be reported in the Chapter 6 & 8 of this thesis. This present chapter presents the frequency distribution of infant and childhood feeding practices. Diet plays a significant aetiological role in the development of enamel defects including enamel hypoplasia and dental fluorosis (Moynihan and Petersen, 2004). In this current study, between 83% and 89% of parents or legal guardians reported that child or ward was exclusively breastfed (EBF) and this rate falls slightly below the WHO/UNICEF recommendation of 90% EBF in children less than 6 months in developing countries (Jones et al., 2003, WHO, 2009). The slightly lower rate

of EBF may be due to traditional beliefs and practices of giving infants water to quench thirst when thirsty or to stop hiccoughs (Davies-Adetugbo, 1997). It may also be due to the majority of women delivering their babies outside health facilities where they were not educated about the importance of exclusive breastfeeding. In addition, it may also be due to the current economic challenges in Nigeria where mothers may be forced to return to full time work causing shorter duration of exclusive breastfeeding and early introduction of complimentary feeding. In Nigeria, there is increasingly early introduction of complimentary food and infants aged 2-3 months are already on transition to solid foods such as rice, yam, beans, cassava, cocoyam, millet, guinea corn, maize products and other food sources like biscuits (Ochonogor, 2013). In accordance with other previous studies (Ifediora et al., 2006, Ochonogor, 2013), water, carbonated drink, liquid milk or yoghurt and tea were the drinks mostly consumed by participants while cooked cassava/yam/maize products followed by rice/beans and soup were foods mostly consumed during weaning. Information about infant and childhood feeding practices was obtained by asking parents or legal guardians to mention drinks and foods consumed by their child or ward during weaning. The responses from them might not be valid due to respondent bias of reporting what they think the interviewer will want to hear. However efforts were made to ensure that they provided valid responses by using open rather than closed questioning and also asking them to mention the commonly consumed drinks and foods. It was not surprising to observe that quite a large number of children consumed tea since tea is grown in Nigeria and it is relatively cheap. Consumption of tea by this large number of children could predispose them to increased sugar consumption and its attendant health problems of dental caries and obesity. Theron et al. (2007) reported that cold carbonated drinks were the third most commonly consumed drink/food item among urban South African children aged 12-24 months. In the urban Ibadan, Nigeria, 16% of children aged 6 to 18 months were given soft drinks a minimum of 1 time per day as a weaning drink (Bankole et al., 2006). Comparison of drink and food consumption of the study participants by areas will be discussed in chapter 6.

The duration of breastfeeding has an influence on child's nutritional status, morbidity and mortality (Susilowati et al., 2010). WHO recommends breastfeeding up to and beyond 2 years (WHO, 2003). Consistent with a previous Nigerian study (Senbanjo et al., 2014) on breastfeeding policy and practices at a paediatric outpatient clinic in Nigeria, over 80% of parents/carers reported that their child/ward stopped breastfeeding after 11 months of age.

This previous Nigerian study (Senbanjo et al., 2014) reported that breastfeeding duration ranged between 1 and 19 months and the mean duration was 11.5 months.

5.5.13 Infant and childhood tooth cleaning behaviours

To estimate the risk of dental fluorosis in young children, F intake also needs to consider tooth brushing with fluoridated dentifrices (Martins et al., 2011a). Young children often ingest a large portion of the toothpaste dispensed on their toothbrush thereby increasing the risk of developing dental fluorosis (Oliveira et al., 2007, Martin et al., 2008). F intake from toothpaste can be assessed quantitatively by measuring the proportion of F retained on the toothbrush and in tooth brushing expectorate (Zohoori et al., 2012). In addition, in large study populations, it can also be assessed by estimating the amount of F toothpaste used based on parents' questionnaire responses (Franzman et al., 2006). This was the method used to quantitatively assess F intake from toothpaste in this present study where mothers were asked to select the diagram that best depicted the amount of toothpaste that their child routinely used from a series of diagrams of toothbrushes holding varying amounts of toothpaste. Similarly, information about infant and childhood tooth cleaning behaviours were obtained from mothers in a previous study (Martins et al., 2011a). Estimating the amount of F intake from toothpaste based on parents' or legal guardians' responses can under or overestimate F toothpaste used or F intake from toothpaste because they might want to give responses that they feel will impress the interviewer especially if they know that the interviewer was a dentist. Also, the temptation of parents or legal guardians to assume that more is better in terms of toothpaste dispensed on toothbrushes might overestimate F toothpaste used. In this present study, the majority of parents or legal guardians reported that study participants brushed their teeth with toothpaste while very few 8 year olds cleaned their teeth with chewing sticks. It was not surprising that many children in this study used toothpaste to clean their teeth because toothpastes are widely available in low cost sachets in the study locations.

In this study, the mean (SD) weight of toothpaste dispensed was 0.54 (0.27) g and 1.51 (0.50) g for 4 and 8 year olds respectively which could be due to the latter age group using more toothpaste than the former due to less control over tooth brushing practices by the parent. The mean (SD) weight of toothpaste dispensed reported for 4 to 6 year old English children (Zohoori et al., 2012) was 0.67 (0.36) g which was higher and lower than that used by 4 year and 8 year olds respectively in this present study. The average amount of toothpaste used per brushing session by 2 to 7 year olds was 0.45 g for 4-year-old children

in Canada (Naccache et al., 1992), 0.36 g for 30-month old English children (Bentley et al., 1999), 0.43 g for 4 to 7-year-old children in Brazil (Pessan et al., 2003) and 0.49 g for 1 to 3 year old Brazilian children (De Almeida et al., 2007). There is no doubt that the differences in the amount of toothpaste used per brushing is due to different tooth brushing practices undertaken by the different age group of study participants. Children who receive less parental supervision may be prone to increased ingestion of toothpaste. There is currently no guideline for the use of fluoridated toothpaste by children in Nigeria, however, the Nigerian Dental Association recommends use of a pea-sized amount of toothpaste and supervised brushing to minimise swallowing for children under 7 years. The mean (SD) weight of toothpaste dispensed in this study was higher than the pea-sized amount (0.25g) recommended by a UK guideline (DoH/BASCD, 2009) for children 3 to 6 year old. In this study over 70% of 4 and 8 year olds used more than 0.25 g of toothpaste during tooth brushing. Efforts must be made to educate parents or guardians on the need to ensure that adequate amount of toothpaste is dispensed on tooth brush of their children/wards per brushing so as to prevent excessive ingestion of F from toothpaste. This is because a previous study (Zohoori et al., 2012) showed that F intake per brushing session was significantly influenced by weight of toothpaste.

About 89% and 95% of 4 and 8 year olds who used toothpaste rinsed their mouth after use while correspondingly, 2% and 0.3% did not rinse or spit out toothpaste after use. This is because young children tend to swallow the toothpaste rather than to rinse or spit them out due to the flavour taste of most toothpastes. Swallowing F toothpaste in early years of life has been postulated to be a risk for dental fluorosis (Mascarenhas and Burt, 1998). Brushing with a F toothpaste should be followed by rinsing procedures which enhance the retention of F (Sjogren and Melin, 2001). Tooth brushing once daily was reported by about 84% and 89% of the parents/legal guardians of 4 and 8 year olds respectively which was at variance with 73.1% reported for 11 to 16 year old Nigerians (Okoye and Ekwueme, 2011). The corresponding figure for tooth brushing twice daily was 15.4% and 11.3% for 4 and 8 year olds respectively which were higher than 7.8% reported for children from southern Nigeria (Ola et al., 2013). However, it was lower than 69% and 76% reported for 4 to 6-year (Zohoori et al., 2012) and 5-year old English (Pendry et al., 2004) children respectively. These differences in the frequencies of daily tooth brushing among these group of children might be due to variations in parental support, supervision and guidance provided to the children.

5.5.14 Prevalence and severity of dental fluorosis and fluoride exposure from drinking and cooking water

High-fluoride drinking water is normally considered to be the major source of ingestible fluoride (Dean, 1934). The relationship between the fluoride concentration in drinking water and dental fluorosis has been studied around the world, and a number of reports have discussed the African context (Fejerskov et al., 1977, Olsson, 1979, Manji et al., 1986). Notably, some papers have reported a high prevalence of dental fluorosis even in areas with a low fluoride content (< 0·5 mg/L) in the drinking water (Manji et al., 1986, Ibrahim et al., 1995, El-Nadeef and Honkala, 1998). These findings have been partly ascribed to food habits such as the consumption of tea (Olsson, 1978, Opinya et al., 1991a) and the use of fluoride-containing trona (*magadi*) (Mabelya et al., 1997, Awadia et al., 2000b). The need for more studies on the relationship between dental fluorosis and fluoride exposure from drinking and cooking water cannot be overemphasized. Information from these studies will further confirm the positive relationship between dental fluorosis and F concentration of water or it will provide a basis for further knowledge concerning other influential factors if this relationship is negative.

In accordance with other previous studies (Zerihun et al., 2006, Wondwossen et al., 2006, Meyer-Lueckel et al., 2011), the prevalence of dental fluorosis was higher among participants who drank or consumed diets cooked with high F concentration water than those who drank or consumed diets cooked with low F concentration water. It was 8.5%, 10% and 22% in the primary teeth of 8 year olds who consumed diets cooked with less than 0.7 ppm, 0.7-1.2 ppm and greater than 1.2 ppm F water respectively. The corresponding figure for the permanent teeth of 8 year olds was 24.5%, 45% and 77.8% respectively. In a study (Firempong et al., 2013) on soluble F levels in drinking water and occurrence of dental fluorosis among Ghanaian children the prevalence of dental fluorosis was 10% and 63% among children who lived in less than 1.0 ppm water F area and greater than 1.2 ppm area respectively. In this present study, there was a very weak though statistically significant positive correlation between F concentration in drinking water and the severity of dental fluorosis in the primary dentition of 4 and 8 year olds. However, there was a weak though statistically significant positive correlation between F concentration in drinking and cooking water and the severity of dental fluorosis in the permanent dentition of 8 year olds. In summary, dental fluorosis severity was related to drinking water for both age groups but only cooking water for permanent teeth in the 8 year-olds. The higher concentrations of F in cooking water as well as greater duration of F exposure from cooking water on the

permanent tooth buds of 8 year-olds compared with the primary tooth buds of 4 year-olds may be the reason for these differences seen in the permanent compared with the primary dentition. Overall, the relationship between F concentrations in water was more pronounced in the permanent teeth of 8 year olds than in primary teeth of 4 and 8 year olds. In addition, a significant proportion of F exposure is prevented by the placental barrier from affecting the early forming primary tooth buds since most of their calcification occurs in-utero while the permanent tooth buds are exposed to F because there is no barrier preventing F from getting to them postnatally.

5.5.15 Conclusions

- The mouth (77.8%) and tooth (mean (SD) number of teeth) (4.0(3.33) prevalence of DDE in the primary teeth was higher among 4 year olds when compared to 8 year olds (64.6% and 2.03(1.91) respectively). It was also higher when compared to permanent teeth of 8 year olds (67.1% and 2.37(2.22) respectively).
- Based on TFI, for 4 year olds, the mouth (5.6%) and tooth (mean (SD) number of teeth) (0.45(1.66) prevalence of dental fluorosis in primary teeth was lower when compared to 8 year olds (9.3% and 0.45(1.66) respectively). It was also lower when compared to permanent teeth of 8 year olds (29.8% and 2.09(3.66) respectively).
- The mouth (10.6%) and tooth (mean(SD) number of teeth) (0.29(1.07) prevalence of dental caries in the primary teeth of 4 year olds was lower when compared to 8 year olds (16.8% and 0.43(1.18) respectively). It was however, higher when compared to permanent teeth of 8 year olds (7.5% and 0.13(0.52) respectively).
- For 4 year olds, respectively 3.3%, 54.3% and 16.6% of them had abnormal child birth, infant/childhood illnesses and were not exclusively breastfed. The corresponding figures for 8 year olds were 1.6%, 54.7% and 10.9%.
- The majority of children brushed their teeth. Of these 16.2% and 70.9% of 4 year olds brushed ≥ twice daily and used > 2.5 g toothpaste respectively. For 8 year olds, 12.4% and 77.2% brushed ≥ twice daily and used > 2.5 g toothpaste respectively.
- Based on TFI, the prevalence of dental fluorosis in primary teeth was 5.9%, 4.3% and 6.3% among 4 year old participants who drank low, moderate and high F concentration water while it was 5.5%, 8.0% and 6.7% for consumption of diets cooked with low, moderate and high F water. There was a weak relationship between the severity of dental fluorosis in primary teeth and F exposure in drinking water among 4 year olds (ρ=0.12; p=0.04).

- Based on TFI, the prevalence of dental fluorosis in primary teeth was 8.5%, 7.1% and 22.1% among 8 year old participants who drank low, moderate and high F concentration water while it was 8.5%, 10.0% and 22.0% for consumption of diets cooked with low, moderate and high F water. There was a weak relationship between the severity of dental fluorosis in primary teeth and F exposure in drinking water among 8 year olds (ρ=0.15; p=0.01).
- Based on TFI, the prevalence of dental fluorosis in permanent teeth was 24.7%, 64.3% and 59.1% among 8 year old participants who drank low, moderate and high F concentration water while it was 24.5%, 45.0% and 77.8% for consumption of diets cooked with low, moderate and high F water. There was a moderate relationship between the severity of dental fluorosis in permanent teeth and F exposure in drinking (ρ=0.28; p=0.001) and cooking (ρ=0.17; p=0.002) water among 8 year olds.

Chapter 6 Phase 2 of the main study – Estimating fluoride exposure.

6.1 Introduction

The role of fluoride (F) in the prevention and control of dental caries is well documented. Most of the global decline of dental caries incidence and prevalence is due to F exposure (Bratthall et al., 1996). Excessive F exposure during tooth development can increase the risk of developing dental fluorosis (Bronckers et al., 2009). Obtaining an estimate of overall F intake and excretion is required to help assess this risk. Knowledge of levels of F intake and excretion is important in planning optimum F therapy for young children. This chapter describes Phase 2 of the main study – the estimate of F exposure in 4 and 8 year old Nigerian children.

The most important metabolic pathway for F elimination from the body is through the urine. Knowledge of F intake, excretion and retention is essential for understanding the biological effects of this ion in humans as well as drive the prevention and treatment of F toxicity (Buzalaf and Whitford, 2011). In children, it is suggested that a total daily F intake of 0.05 – 0.07 mg per kg of body weight (mg/kg bw/day) is optimal to provide a dental health benefit (Burt, 1992, American Academy of Pediatics Committee on Nutrition, 1995) with the tolerable upper intake level for children to minimise the risk of dental fluorosis defined by US Institute of Medicine as daily intake of 0.1mgF/kg bw/day (Institute of Medicine, 1999). Urinary F excretion rate in optimal fluoridated communities ranges from 11.6 to 19.8 μg/h (Villa et al. 2000; Zohoori et al. 2000). Urinary F excretion rate of 0.019 to 0.026 mg/kg bw/day in children between 1 to 14 years could be considered as an indicator of "optimal" F intake (WHO, 2014). Dental professionals should evaluate daily F intake as well as urinary F excretion of young child patients before recommending any F use which may involve systemic ingestion of F. A study of the F intake and urinary F excretion of 4 and 8-year olds is described in this chapter

6.2 Aim

To estimate F exposure among a subgroup of the Phase 1 study participants by measuring their F intake from diet and toothpaste, their urinary F excretion and estimating body F retention.

6.3 Materials and methods

6.3.1 Introduction

In Phase 1 of the main study, clinical dental examination of defects and photographic examination of dental fluorosis among study participants were conducted to provide information on prevalence and severity of developmental enamel defects. In addition, information on illnesses, tooth cleaning and feeding practices during infancy or childhood was obtained. In Phase 2, information on F exposure from diets and toothpaste and urinary F excretion was collected from a subsample of Phase 1 study participants to estimate F exposure and investigate any relationship between F exposure and the occurrence of developmental enamel defects.

6.3.2 Phase 2 study participants recruitment and sample size

Using nurseries (4 year-olds) and primary schools (8 year-olds) in lower (<0.2ppmF) and higher (0.6≥ppmF) water F areas in rural and urban communities in south western Nigeria, a subsample of 144 study participants from the 624 study participants (302 4-year-olds) and (322 8-year-olds) who took part in Phase 1 of the study were randomly recruited but was attended to on first come first recruit basis. The original letter of invitation sent to parents/legal guardians seeking permission to allow their child/ward to participate included information about Phase 2 and Phase 3 of the study (See Appendix F) and a 20% subsample (n=128) was recruited for the two later Phases of the research. To allow for attrition, 144 children comprising 72 four year olds and 72 eight year olds and their parents or legal guardians were planned to be recruited (Figure 6.1) using an enrolment ratio of 8:1 from public and private primary schools based on Nigerian school enrolment records(UNESCO, 2012). Figure 6.1 shows the planned and actual sampling numbers and ratios of participants for Phases 2 and 3 recruited from nurseries and primary schools.

6.3.3 Preparatory work in the UK

6.3.3.1 Training on risk and BioCOSHH assessments and F concentration measurements

The researcher attended training on risk assessment of biological and chemical hazards in the laboratory of the Newcastle University. Training on F concentration measurements for biological (e.g. urine) and non-biological (e.g. drinks and foods) samples using a F-ion-selective-electrode (F-ISE) by direct and indirect methods was also undertaken using the F analysis training manual.

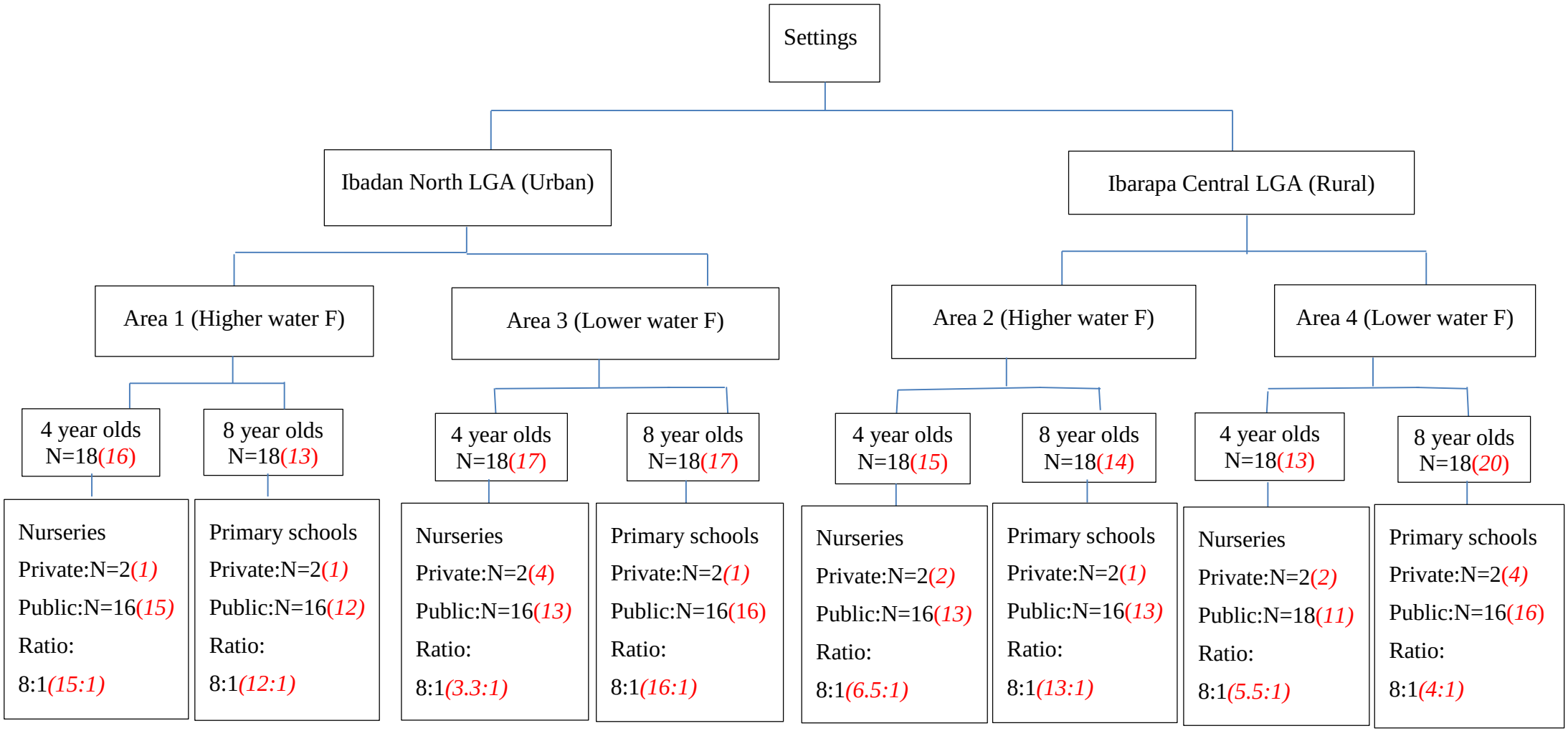

Figure 6-1: Sampling of study participants for Phases 2 and 3 from nurseries and primary schools in urban and rural higher (≥0.6 ppmF) and lower (≤0.2 ppmF) water F concentration in community water supply.

Notes: Actual recruitment no. (N=144) and ratio (8:1) – numbers in black; No. completing study (N=125) and ratio (1:3.3 – 1:16) in red, italicised and in parenthesis.

6.3.3.2 Development of Food Frequency Questionnaire (FFQ) and 24 hour urine collection data sheet

The FFQ (Appendix S) and 24 hour urine collection data sheet (Appendix T) were developed after several revisions. The FFQ consist of questions on type, frequency and amount of food and drinks consumed by the study participants. The urine data sheet contained questions about time when first and last urine were voided and amount of urine voided by the study participants.

6.3.3.3 Authorization

Permission to import food and drink samples from Nigeria to the UK for F concentration measurement at the F research laboratory, Newcastle University, United Kingdom was obtained from Department for Environment Food and Rural Affairs (DEFRA) (Appendix M).

6.3.4 Preparatory work in Nigeria

6.3.4.1 Training of the research team

This took place during a 2 week period in January 2013, just prior to the start of the fieldwork phase of the study. The nutritionist was trained by the researcher for two days on how to administer the FFQ to parents/legal guardians of study participants. During the training, the nutritionist administered the questionnaire to three parent volunteers. The nutritionist was also trained on how to instruct parents/legal guardians to collect samples of drink and food. Instructions included how the food and drink samples were placed into the food and drink collection receptacles provided. Furthermore, the researcher trained the laboratory technician for 2 days on how to measure F concentration in urine using F-ISE by the direct method using the F analysis training manual (Omid et al., 2011). In addition, the two research assistants were trained by the researcher on how to collect food and drink samples from homes of study participants and store them in the research freezer at the Oral Pathology Laboratory, Dental School, University of Ibadan, Nigeria. The researcher also trained the research assistant on how to collect, measure and transport the 24 hour urine of study participants to the laboratory using the training guide.

6.3.4.2 Scheduling and setting up and monitoring of fieldwork and milestones

A work schedule was drawn up (Appendix L) to cover the data collection periods (January - June, 2013). Fieldwork commenced in an urban area, moved to a rural area then another urban area and finally the other rural area. On each day of data collection, the research vehicle was used to convey the research team, materials and equipment from the Dental

School University of Ibadan, Nigeria to the selected nurseries and primary schools. Before departure to study site, all the materials and equipment were checked to ensure that they were complete and in good condition. Once the materials and equipment had been transported to the designated dental examination room in the school, the FFQs and food and drink sample receptacles were given to the nutritionist for data collection just at the room entrance. The compiled list of Phase 2 study participants was given to the research assistants to identify children in their classes and prepare them for the 24 hour urine collection the following day.

6.3.5 Data collection

6.3.5.1 Anthropometric data collection

To determine the nutritional status of each child, anthropometric measurements such as their height and weight were recorded on data collection sheet (Appendix N) in a school classroom. The height of each subject was measured without shoes or head-wear using a single stadiometer (DE56618903; ADE Germany) to the nearest 0.5 cm while weight without heavy clothes and shoes was measured in kg (to the nearest 0.5kg) using an electronic digital scale (SOEHNLE, Slim Design Linea, Germany). The same weight and height scales were used throughout the study to avoid any possible measurement errors. The accuracy of the stadiometer and the scale was periodically verified using a reference height and weight. All measurements were taken by the trained record clerk. Age on the day of examination was calculated. To determine their nutritional status the 4 year olds were classified for stunting and wasting using the Waterlow classification (Waterlow et al., 1977) (Table 6.1) while 8 year olds were classified for stunting and malnutrition using the Waterlow classification (Table 6.1) and Gomez classification (Gomez et al., 1956) (Table 6.2) respectively. Wasting could not be calculated for 8 year olds because there was no weight for height for 5 to 10 years in the WHO Child Growth Standards.

Interpretation	% Weight for Height (Wasting)	% Height for Age (Stunting)
Normal	> 90	> 95
Mild	80 – 90	90 – 95
Moderate	70 – 80	85 – 90
Severe	< 70	< 85

Table 6-1: Waterlow classification for stunting and wasting. Waterlow et al. (1977)

Stunting and wasting values were calculated from the WHO Child Growth Standards (WHO, 2006b) using height for age and weight for height respectively. The percentage height for age for each participant was calculated by dividing the height of participants by

the height of normal child of the same age multiplied by 100 while the percentage weight for height was calculated by dividing weight of participants by the weight of normal child of the same height multiplied by 100.

Interpretation	Percentage of reference weight for age
Normal	90 – 110%
Grade I: Mild malnutrition	75 – 89%
Grade II: Moderate malnutrition	60 – 74%
Grade III: Severe malnutrition	< 60%

Table 6-2: Gomez classification for malnutrition. Gomez et al. (1956)

Malnutrition was calculated by comparing a child's weight to that of a normal child (50^{th} percentile of the same age) using the weight for age WHO Child Growth Standards. The % weight for age of the participants was calculated by dividing weight of participants by weight of normal child of same age multiplied by 100.

6.3.5.2 Food Frequency Questionnaire (FFQ)

Parents or legal guardians were interviewed by the trained nutritionist using a FFQ (Appendix S) to obtain information on the current amount and frequency of food and drink consumption by study participants. Data were recorded on the FFQ and the 14 drink and 17 food groups for which there were data were identified (Table 6.3).

6.3.6 Sample collection, preparation and storage in Nigeria

6.3.6.1 Drink and food samples

- **Home-made drinks and foods**

After interviewing parents/legal guardians on patterns of drink and food consumption, the nutritionist provided them with drink and food collection receptacles (Ziploc bags, universal tubes and polystyrene bowls) labelled with child/ward's study number and school identification codes. In addition, the nutritionist instructed parents/legal guardians to provide a serving spoon of any home-made food and half tea cup of any home-prepared drink consumed by study participants, placing samples into the ziploc bags and 30 ml universal tubes respectively for the research assistants to pick up. Samples were taken to the Oral Pathology Laboratory Faculty of Dentistry, University of Ibadan, Nigeria and stored in a – 20^{o}C freezer by the trained laboratory technician. Each home-made drink and food sample provided was homogenised using a blender and then divided into 2 aliquots. Each drink aliquot was placed in a bijou bottle while each food aliquot was placed in a Ziploc

bag and stored at -20ºC in the Oral Pathology Laboratory prior to transportation to the UK for F analysis.

Code	Drink group (DG)	Code	Food group (FG)
DG1	Tap water	FG1	Cooked vegetables
DG2	Well/borehole water	FG2	Steamed vegetables
DG3	Bottled/sachet water	FG3	Raw fruits
DG4	Black tea	FG4	Steamed fruits
DG5	Herbal tea	FG5	Soup
DG6	Hot drinks (Chocolate)	FG6	Bread
DG7	Liquid milk or yoghurt	FG7	Cooked pasta/noodles/spaghetti
DG8	Powdered milk	FG8	Cooked rice and beans dishes
DG9	Sugared ready to drink	FG9	Steamed rice and beans dishes
DG10	Sugar-free ready to drink	FG10	Cooked fish or sea foods
DG11	Liquid fruit concentrate prepared at home - Sugared	FG11	Fried/roasted fish or sea foods
DG12	Liquid fruit concentrate prepared at home – Sugar-free	FG12	Breakfast cereals
DG13	Powdered fruit concentrate prepared at home	FG13	Cooked meat or meat products
DG14	Carbonated drink	FG14	Fried/roasted meat or meat or meat products
		FG15	Cooked yam/cassava/maize
		FG16	Fried/roasted yam/cassava/maize
		FG17	Confectioneries

Table 6-3: Codes used for drink and food groups in the Food Frequency Questionnaire.

- ***Ready-to-drink and ready-to-eat samples***

After completion of the FFQs for a particular school (Nursery or Primary), a list of the most frequently consumed (four or more times weekly) ready-to-eat drinks and ready-to-eat-foods were drawn up from the questionnaires. The most frequently consumed items were then purchased from commercial stores in the locality based on decreasing frequency according to the total allocated budget for purchasing of items for the area. Each drink or food purchased was divided into 2 aliquots; drink aliquots were placed in a bijou bottle while food aliquots were placed in a Ziploc bag and then stored in the – 20^{O}C freezer, prior to transportation to the UK for F analysis.

6.3.6.2 Toothpaste samples

A list of all the types of toothpaste used by study participants whose parents/legal guardians reported that they brushed their teeth with F toothpaste was derived from the Phase 1 questionnaires. Nine toothpastes were identified from this list and purchased from commercial stores in Ibadan, Nigeria: Close up Deep action, Olive triple action, Maccleans

complete care, Mymy dental fresh, Promise, Oral B, Maxam, Darbul and Max. The toothpastes were kept at room temperature in a cupboard at the Oral Pathology Laboratory prior to transportation to the UK for F analysis.

6.3.6.3 Twenty four hour urine data and sample collection

- **Preparation for urine collection**

To enable the collection of 24 hour urine samples, urine collection receptacles (plastic funnel, bottle and cup) for home use were given to each parent/legal guardian by the research assistants when they provided information about frequency of food and drink consumption. Each parent/legal guardian and child/ward were also shown another set of collection receptacles that was used to collect urine at school at the start of the 24 hour collection period. The nutritionist went through the steps needed for 24 hour urine collection (Appendix J) with each parent/legal guardian and child/ward to ensure that they understood the details and importance of the urine collection. Emphasis was laid on the efficient collection of the urine and the cooperation of the parent/legal guardians and their child/ward. The nutritionist instructed the parents/legal guardians to keep the home-collected urine in a safe place, preferably their toilet, for collection by the research assistant the following day.

- ***Urine Collection at home***

The time when study participants voided their first urine after waking up from sleep in the morning on Day 1 was recorded or noted by parents or legal guardians but the first urine sample was not collected. Thereafter, parents/legal guardians were requested to closely monitor their child or ward, encouraging them to void urine just before going to nursery or school. Upon return home after school, children were encouraged to pass urine immediately and they were closely monitored and encouraged to pass urine just before going to bed. The first urine voided on Day 2 just when the child arose from bed was also collected and recorded. The 24 hour urine sample collected was then kept in their toilet until it was collected by the research assistant on Day 2. The parents/legal guardians were always reminded through a phone call from the research assistant to ensure that these tasks were carried out efficiently. During this phone call, they were also asked the times that participants voided first and last urine and these times were recorded into the urine data sheet (Appendix T). Upon collection, a private interview was also conducted with the parent/guardian to confirm the correct urine collection procedure had been followed.

- **Urine Collection in the school**

On Day 1 of urine collection in nursery- and primary schools, each participant was shown his/her personal labelled urine collectibles (plastic cup, funnel and bottle) which were kept at the corridor of the toilet. Each child was told to inform their class teacher whenever they wanted to urinate. The teacher then summoned the research assistant to assist them to collect their urine using their own labelled plastic cup, funnel and bottle. Before going on lunch break and going home each child was encouraged to urinate using the urine collecting receptacles. The urine samples collected in the nursery- and primary- schools were kept in a locked room in the nursery or school premises, prior to being transported to the laboratory for measurement of volume.

- ***Pre-analysis preparation and storage of urine***

For each participant, the urine collected at home was pooled together with urine collected in the nursery or primary school and its volume measured using a measuring cylinder. The volume was recorded on the urine data sheet (Appendix T) and two aliquots of 7 ml urine samples were dispensed into labelled bijou bottles for F concentration measurement. The 2 aliquots were then stored at – 20^{o}C before F analysis.

6.3.7 Urine sample preparation, F analysis and disposal in Nigeria

The frozen urine samples were defrosted on the bench in the Oral Pathology Laboratory Faculty of Dentistry, University of Ibadan, Nigeria at room temperature just prior to F analysis. Urine samples of each of the participants were then measured in triplicate at room temperature using F-ISE (Model 9409 Thermo Orion, USA) and meter (Model 720) by direct method after adding TISAB III in the proportion of 1:10 (v/v) as stated in the SOP. Prior to measuring F concentration of samples, the electrode was calibrated using a series of standards prepared by adding TISAB III in a proportion of 1:10 (v/v). Concentrations of F standards were chosen to ensure that they covered the range of the expected sample concentrations (Martínez-Mier et al., 2011).

The reliability of the urine F analytical methods was examined by re-analysing 10% of the urine samples for their F concentration. The remaining urine samples were disposed of by a microbiologist specialist in line with the laboratory's urine disposal guideline. The bijous containing urine were placed in concentrated NaCIO solution for more than 2 hours before they were washed and placed into black plastic bags and disposed of in the laboratory's rubbish disposal system.

6.3.8 Transport of drink, food and toothpaste samples to the UK

Upon completion of the field work in all areas, one set of the drink and food samples in bijou bottles and zip lock bags respectively remained in the -20^{o}C freezer in Nigeria as a back-up until sample analysis in UK was completed. The other set of the drink and food samples in bijou bottles and zip lock bags respectively and toothpaste samples were then transported to the Fluoride Research laboratory Newcastle University, UK using dry ice. Food and drink samples were stored at -20^{o}C in the fluoride research laboratory at Newcastle University while the toothpaste samples were kept at room temperature prior to F analysis.

6.3.9 Preparation and laboratory analysis of samples in the UK

6.3.9.1 Combination and categorization of drink and food samples

A list of all the home-made and purchased drink and food samples collected in each area was drawn up from the label of each bijou and zip lock bag and then categorised into the Food Frequency Questionnaire (FFQ) Drink (DG) and Food (FG) groups (14 DG and 17 FG. Single composition samples were classified simply into their type of FFQ drink and food group while those drink and food samples containing mixed solid/semisolid/liquids were classified based on their main composition. This resulted in 3 DG (hot drinks, milk/yoghurt & carbonated drinks) and 8 FG (cooked vegetable dishes, soup, bread, noodles/pasta, rice & beans dishes, cereal mixed with water and milk, cassava/yam/maize products and confectioneries) being identified. The drink and food samples were then placed into plastic bags according to their drink or food group and then separately placed in a large plastic bag labelled with the Area (1-4) where they were collected. Table 6.4 summarises this categorisation and the number of analyses required to produce a value per Area, where a sample was collected.

Each food and drink sample in the labelled food and drink group bag for each Area was weighed and the weight recorded. For drink samples, the weight of an empty bijou was subtracted from the weight of the drink and the bijou. Similarly, for food samples, the weight of the empty zip lock bag was subtracted from the weight of the food and the bag. The smallest weight of drink or food sample recorded was a soup which weighed 8.2g, and this measurement guided the strategy for F analysis of samples.

Drink and Food groups*	Number of samples collected					
	Area 1 (Urban, Higher F)	Area 2 (Rural Higher F)	Area 3 (Urban, Lower F)	Area 4 (Rural, Lower F)	Total number of samples	No of F analyses required
DG6	5	0	1	0	6	2
DG7	2	2	1	2	7	4
DG14	0	0	2	0	2	1
FG1	12	20	23	36	91	4
FG5	11	7	11	14	43	4
FG6	12	1	12	2	27	4
FG7	6	1	8	2	17	4
FG8	56	50	47	63	216	4
FG12	2	0	0	0	2	1
FG15	32	42	53	59	186	4
FG17	6	5	4	2	17	4
Total	**144**	**128**	**162**	**180**	**614**	**36**

Table 6-4: Summary of categorisation of food and drink groups according to study Areas and derived numbers of combined samples ready for F analysis. *Note: * See Table 6.3 for codes used for drink and food groups*

6.3.9.2 Preparation of drink samples

Single sample: The frozen drink samples were defrosted at room temperature by leaving them on the bench for some hours. For single liquid samples (i.e. Drink groups DG6 and DG7 for Area 3 in Table 6.4); after vortexing using a vortex mixer (Stuart Scientific Autovortex Mixer SA2 UK) to thoroughly mix the sample, two 10g aliquots of the single (1 sample) drink sample were pipetted into two labelled universal bottles and thoroughly mixed again by vortexing. One 10g aliquot was stored in a fridge at 4^{o}C prior to F analysis while the other was stored at -20^{o}C as a back-up. Multiple samples: Where more than one liquid sample contributed to a combined sample (see Table 6.4) after vortexing, 10g of each drink sample was combined together after being separately pipetted into a labelled universal bottle and the combined sample thoroughly mixed by vortexing. Two aliquots, each of 10g of the final combined vortexed sample were then pipetted into 2 labelled universal bottle and one 10g aliquot stored at 4^{o}C prior to F analysis while the other was stored at -20^{o}C as a back-up.

6.3.9.3 Preparation of food samples

For single food samples, (i.e. FG6 and FG7 for Area 2 in Table 6.1), after defrosting, the food sample was homogenised using appropriate amount of De-ionized Distilled Water – DDW and a two 10g aliquots of the single food sample were dispensed into two well labelled zip lock bags (One stored at 4^{o}C prior to F analysis, the other at -20^{o}C as a back-up).

For multiple food samples, (i.e. FG1 for all Areas in Table 6.4), after defrosting, 10g of each food sample was combined together in a homogeniser (Thermomix TM31 blender manufactured by Vorwek, Germany) and thoroughly mixed and homogenised using appropriate amount of De-ionized Distilled Water - DDW. Two 10g aliquots of the combined homogenised food sample were dispensed into two labelled ziploc bags (One stored at 4^{o}C prior to F analysis, the other at -20^{o}C as a back-up).

6.3.9.4 Preparation of toothpaste samples

Two cm of each toothpaste was discarded and then 1 g from each toothpaste was measured for F analysis.

6.3.10 Analysis of F concentration of samples in the UK

The F concentration of each drink, food and toothpaste sample was carried out in triplicate using a F-ISE after adding TISAB III in the proportion of 1:10 (v/v) at room temperature by Dr Narges Omid. For non-milk based drinks a direct method (Martínez-Mier et al., 2011) was used, while for food and milk-based drink samples an indirect method, the HMDS acid-diffusion technique (Whitford, 1996a, Venkateswarlu and Vogel, 1996). For toothpaste samples, 10 ml of DDW was added to each 1 gm toothpaste sample (10:1) and then stirred for 5 minutes. The slurry was then further diluted by adding 10 gm of DDW to 0.1 gm of slurry (1:100) (Cochran et al., 2004b). Toothpastes containing SMFP were treated with acid buffer and acid phosphatase and incubated for 3 hours for 37^{o}C (Duckworth et al., 1991).

Regarding disposal of samples, drink and food samples were disposed of in the laboratory sink while toothpaste samples were placed into black plastic bags and disposed into the laboratory rubbish disposal system according to the Newcastle University's Health and Safety and Good Laboratory Practice protocols.

6.3.11 Quality control of the analytical methods

The reliability of the F analytical methods was examined by re-analysing 10% of all samples including drink, food and toothpaste for their F concentration. The validity of the fluoride analytical methods was also checked by adding a known amount of fluoride to 10% of samples. The recovery of the added fluoride was determined by measuring the fluoride concentration of the samples with and without the addition of F.

6.3.12 Collection of data on Fluoride intake from tooth brushing

Information about type of toothpaste, active ingredient in the toothpaste and amount of F in the toothpaste (ppm) were obtained from the toothpaste labels. The amount of toothpaste dispensed onto a toothbrush by the study participants per brushing (g) was determined by asking their parents/legal guardians to point to the amount used from a diagram in the questionnaire (Appendix O) used in Phase 1 of the study. Information on the actual F concentration of the toothpaste samples (μg/g) was obtained from the F analysis of toothpaste samples (see Section 6.3.10).

6.3.13 Data management and handling

6.3.13.1 In Nigeria

The anthropometric data and recordings in the laboratory book were respectively checked on daily basis before leaving the study location and laboratory for errors and omissions. The FFQs were checked against the field work diary and the data were collated and verified for errors and omissions daily before leaving the study location. Where there were errors and/or omissions, parents/legal guardians of study participants were re-called and accurate responses were confirmed.

6.3.13.2 In the UK

In the UK, the amount of each type of drink (ml) and food (g) consumed per serving and the amount (ml or g) consumed daily were calculated on the FFQ from the responses using a red pen. The calculated amount for each drink (ml) and food (g) consumed daily were then recorded on this questionnaire using a green pen to ensure clarity. The values recorded with the green pen were entered into an excel spread sheet. Prior to these calculations, to check their validity and reliability, 10% of the questionnaires were randomly selected by an independent researcher and photocopied. Then the same calculations for these questionnaires were undertaken using the same process as before. The first and second sets of data entry for this subset of 10% of the questionnaires were compared and no differences were seen in the calculated amounts. Further checks for the validity and reliability of the data entry was undertaken by re-entering a randomly selected 10% of the anthropometric data sheets and the FFQ questionnaires into an excel spread sheet and comparing the 1st and 2nd data sets; again, no differences were found.

6.3.14 Deriving the outcome variables and data analysis

6.3.14.1 Deriving the F intake outcome variables

The following relevant F intake outcome measures were derived:

1. **Daily Dietary Fluoride Intake (DDFI)**: Each child's F intake according to each drink and food group was calculated by multiplying its F concentration (mg/kg) by the amount (g) consumed per day. The overall DDFI from drinks and food was calculated by summing the F intakes from each drink and food group.
2. **Daily fluoride intake from tooth brushing with fluoride toothpaste**: For each child who brushed teeth with fluoride toothpaste, the F dispensed on toothbrush per brushing (μg/brushing) was estimated by multiplying the toothpaste's F concentration (μg/g) by the amount used per brushing (g). The F ingestion from toothpaste per brushing (μg/brushing) was estimated based on the mean proportion of toothpaste ingestion per tooth brushing session among 4 to 6 year olds in England (41%) (Zocor et al. 2012), therefore each value for F dispensed on toothbrush per brushing was multiplied by 41% and then the number of brushings per day. The estimated F intake from toothpaste ingestion per day was expressed on a body weight basis (μg/kgbw/day) by dividing F intake from toothpaste ingestion per day (μg/day) by weight (kg).
3. **Total daily F intake (TDFI):** For each child the TDFI was calculated by adding the DDFI and the daily F intake from tooth brushing either in mg/day or mg/kg bw/day.
4. **Estimated relative contribution (%) of different dietary F sources to total daily dietary fluoride intake**: This was calculated by dividing F intake from different dietary sources of fluoride by the total daily dietary F intake.
5. **Estimated relative contribution (%) of different F sources (i.e. diets and toothpaste) to total daily fluoride intake**: This was calculated by dividing the F intake from the two sources of fluoride (diet and toothpaste) by the total daily F intake.

6.3.14.2 Deriving F excretion outcome variables

To validate the 24h urine sample collections their completeness was assessed by calculating the urinary flow rate and comparing it with the WHO reference ranges (WHO 2014). A urinary flow rate of less than 5ml/hr for children younger than 6 years and less than 9ml/hr for children 6 years and older was regarded as incomplete (WHO 2014). Therefore, study

participants whose urine fell into this category were excluded. The following urinary excretion outcome variables were derived from the urine data:

1. **Corrected urine volume** (for 24 hours): Each urine volume was corrected for 24 hours by dividing the total urine volume (ml) by the duration of collection of urine (min) and then multiplying this value by 1440, (where 1440 is the number of minutes in 24 hours). The duration of urine collection in minutes was also calculated in the same way.

   $$\text{Corrected urine volume (ml/24-h)} = \frac{\text{Total urine volume (ml)}}{\text{Duration of collection (min)}} \times 1440$$

2. **Daily urinary flow rate** (DUFR): The urine flow rate (ml/h) was calculated by dividing the corrected urine volume (ml) by 24 h.
3. **Daily urinary fluoride excretion** (DUFE): The urinary F excretion (mg/day) was calculated by dividing the corrected urine volume by 1000 and then multiplying it by the F concentration in urine (mg). The urinary F excretion (in mg/kg body weight) was calculated by dividing urinary F excretion (mg/day) by child/ward's weight (kg).

   $$\text{Urinary F Excretion (mg/day)} = \frac{\text{Corrected Urine volume (ml)}}{1000} \times \text{F concentration (mg)}$$

   $$\text{Urinary F Excretion (mg/kg body weight/day)} = \frac{\frac{\text{Corrected urine volume (ml)}}{1000} \times \text{F concentration (mg)}}{\text{Child/ward's Weight (kg)}}$$

4. **Fractional urinary fluoride excretion** (FUFE): This was estimated by dividing the total urinary fluoride excretion by total daily fluoride intake (TDFI).

   $$\text{FUFE (\%)} = \frac{\text{Urinary Fluoride Excretion}}{\text{Total Daily Fluoride Intake}} \times 100\%$$

5. **Total daily fluoride retention** (TDFR): This was calculated by subtracting total daily F excretion (TDFE) (mg/day or mg/kg/day) from the total daily F intake (TDFI) (mg/day or mg/kg/day). However, the total daily F excretion (TDFE) is calculated from adding daily urinary F excretion (DUFE) and daily faecal F excretion (DFFE) i.e. TDFE = DUFE + DFFE. The daily faecal F excretion is estimated as 10% of the total daily F intake (TDFI) i.e. 0.1 x TDFI. Therefore, the TDFR = TDFI-(DUFE + 0.1 x TDFI).

6.3.15 Data analysis

Data were exported into Statistical Package of Social Sciences (SPSS) software from the excel spreadsheet for analysis. Descriptive analyses were presented as proportions, mean

(SD) and median (minimum, maximum) of the variables. Student t-test was used to compare differences between the means of 2 groups while 1-way ANOVA and Tukey Post Hoc Tests were used to compare differences between the means for more than 2 groups at a 5% level of statistical significance. For data that were not normally distributed, Mann-Whitney test was used to compare the mean ranks of the 2 independent groups at $p<0.05$. Correlation analyses were undertaken to explore the direction and strength of the relationships between relevant variables at $p<0.05$.

6.4 Results

6.4.1 Recruitment

Of the 144 parents/legal guardians who consented to participate and allow their children/wards to participate in Phase 2, 125 (86.8%) completed a FFQ and provided valid 24 hour urine samples (Table 6.5). The participation ratio was 8:1 for all clusters. The completion rate for Phase 2 was between 1:3.3 (for Area 3: – 4 year olds) and 1:16 (for Area 3: - 8 year olds (Figure 6.1 and Table 6.5).

Study participant characteristics	**4 years (n=71) No. (%)**	**8 years (n=73) No. (%)**	**Total (n=144) No. (%)**
Completed the interviewer-administered FFQ	68 (95.8)	70 (95.9)	138 (95.8)
Provided breakfast, lunch and dinner samples	66 (93.0)	69 (94.5)	135 (93.8)
Provided 24 hour urine samples	65 (91.5)	67 (91.8)	132 (91.7)
Urine samples meeting validation criteria for inclusion*	61 (85.9)	64 (87.7)	125 (86.8)
Completed FFQ and provided valid 24-hr urine samples	61 (85.9)	64 (87.7)	125 (86.8)

Table 6-5: Data collection characteristics of study participants in Phase 2 (n=144).

*Notes: * 4 year olds (Urine flow rate 5-160 ml/hour; 8 year olds (Urine flow rate 9-300 ml/hour) WHO (2014).*

6.4.2 Quality control of analytical methods (F concentration measurement in drink, food, toothpaste and urine samples)

Ten percent of the 7 drink and 29 food samples were re-analysed for F concentration and the mean difference (range) was 0.014 (0.004 – 0.033) μgF/ml. The mean recovery of a known amount of F added to these samples was 94% (Range; 90% to 96%). Similarly, 10% of the 9 toothpaste samples purchased from commercial stores in Ibadan were re-analysed and the mean test- to re-test difference was 0.006 ugF/ml with a range of 0.004 to 0.009 ugF/ml. The mean recovery of the known amount of F added to the 2 toothpaste samples was 96%. In addition, 10% of the 144 urine samples were re-analysed for F concentration

and the mean difference (range) between first and second analysis was 0.007 (-0.247 to 0.233 μgF/ml) (p=0.95).

6.4.3 F concentration of drink and food samples and the strategy for deriving F intakes

6.4.3.1 Measured F concentration of drink and food samples

Waters used for cooking and drinking were collected for all Phase 1 participants and the results reported in Chapter 5 (Section 5.4.3). For Phase 2 participants, all other drink and food samples consumed as reported in the FFQs were intended for F analysis. From the summary of the samples collected by study area, shown in Table 6.4 it was observed that samples were not obtained from all drink and food groups consumed. Therefore, the UK database developed by Newcastle University and Teesside University (Maguire et al., 2012), was considered to provide the best estimate of F contents of the missing drink and food group samples. Those drink and food samples which were obtained were pooled and analysed for F as one pooled sample for each area, resulting in 7 samples from 3 drink groups and 29 samples from 8 food groups being analysed for F concentration (Appendix BB). The F concentration in drink samples ranged from 0.026 μg/ml in Area 2 to 0.487 μg/ml in Area 3 while the F concentration in food samples ranged from 0.118 to 0.902 μg/g in Area 1; 0.05 to 6.117 μg/g in Area 2; 0.06 to 0.757 μg/g in Area 3 and; 0.069 to 1.22 μg/g in Area 4 (Appendix BB). Soup consumed in Area 2 contained the highest F concentration of 6.117μg/g while milk/yoghurt in Area 3 contained the lowest F concentration of 0.013 μg/ml.

6.4.3.2 Estimated total amount of drink (ml) and food (g) consumed by 4 and 8 year olds using FFQ

All drink and food group samples consumed by the study participants as reported in the FFQ were not obtained from parents or legal guardians. Therefore, a UK database (Maguire et al., 2012) was used, according to the following strategy, to estimate F concentration of the drink and food group samples that were not obtained. Appendix BC presents the total amount of drink (ml) and food (g) consumed by the 125 four and eight year olds based on the results of the FFQ. Between 1 and 121 participants consumed between a total of 0.2 litres of liquid fruit concentrate (DG12 - 1 participant) and a total of 148.4 litres of well/borehole water (DG2 - between 102 participants). One hundred and eighteen participants consumed a total of 16.7 litres ml of hot chocolate made with drinking water (DG7) while 7 participants consumed 0.3 litres of ready to drink as purchased – sugar-free (DG10). Regarding solid foods, no participant consumed fried or roasted cassava or yam or

maize products (FG16) but between 1 and 125 participants consumed the other food groups. The amount of foods consumed by the 125 participants ranged from a total of 0.002 kg for steamed fruits (FG4 - 1 participant) to 62.0 kg for cooked rice and beans dishes (FG8 – between 125 participants). Based on these preliminary analyses, drink and food types consumed by less than 10 participants in Appendix BC were ignored in the subsequent analysis.

After combining the 3 types of drinking water (DG1-DG3) and ignoring drink and food types consumed by less than 10 participants, the total amount of drink (ml) and food (g) consumed by the 125 study participants (Appendix BD) shows that at the high end of consumption, 163 participants consumed a total of 234.6 litres of tap/borehole/bottled water (95 consumed one type of water, 24 drank 2 types and 6 participants drank the 3 water types). Conversely, overall, 37 participants consumed 2.3 litres of sugared liquid fruit concentrate made with drinking water (DG12) daily. Regarding foods, 111 and 121 participants consumed 13.6 litres of liquid milk or yoghurt and 7.6 kilograms of cooked vegetables respectively. Powdered milk (0.9 kg), raw fruits (25.7 kg) and fried meat (0.2 kg) was consumed by 63, 98 and 12 participants respectively. Ninety five participants consumed 8.2 litres of black tea and the source of information for F concentration was from both F concentration measurement of the individual drinking water collected in this present study and the UK F database.

*6.4.3.3 Estimated F concentration of all drinks (µg/ml) and foods (µg/g) consumed by 4 and 8 year olds based on adopted strategy by area**

To complete the process for deriving the data for F intakes, Appendix BE shows final F concentration of all drinks (µg/ml) and foods (µg/g) consumed by 4 and 8 year olds based on the adopted F analysis strategy by area. As Appendix BE describes the sources of information for F concentration varied according to the drink or food group; most were based on samples analysis from the present study (Appendix BB) but some were based on the UK F database. For example, for the drink groups, the mean F concentration of individual drinking waters collected was used to represent the F concentrations of drinking water, herbal tea, hot drinks, liquid and powdered fruit concentrate made with drinking water. The F concentrations of black tea for all Areas were all based on the UK F database for corresponding water F areas (See Appendix BB).

6.4.4 F Analysis of toothpaste samples and labelling information

Table 6.6 shows the F concentration measurement (ppm) of the 9 toothpaste samples. These ranged from 178 μg/g (Max) to 1305 μg/g (Close up Deep Action) with 6 containing more than 1000 ppm F. The F concentration (ppm or %) was not specified on 3 toothpaste labels and 5 recorded containing sodium fluoride while 3 recorded sodium monofluorophosphate.

Brand names of toothpaste	**Type of toothpaste fluoride on toothpaste tube/label**	**Labelled F concentration (in ppm or %) on toothpaste tube/label**	**Analysed F concentration of tooth samples (ppm) analysed in the present study**
Close up deep action	NaF	1450ppm	1305
Olive triple action	NaF	0.32% (1450ppm)	1289
Macleans complete care	NaF	0.306% (1386ppm)	1278
Mymy dental fresh	NaMFP	0.76% (1000ppm)	1199
Promise	NaF	1450ppm	1171
Oral B	NaF	1100ppm	1054
Maxam	NaMFP	Not specified	990
Darbul herbal	NaMFP	Not specified	335
Max	Not specified	Not specified	178

Table 6-6: Labelled and analysed Fluoride concentration (ppm or %) of commercially available toothpaste samples commonly used by 4 and 8 year olds and information on their labels.

6.4.5 Fluoride exposure - Four year old study participants

6.4.5.1 Anthropometric and nutritional status data

The mean (SD) height, weight and BMI for the 4 year olds (n=61) was 1.02 (0.07) m, 15.50 (1.99) kg and 15.02 (2.27) kg/m^2 respectively (Table 6.7). There was no statistically significant difference in height, weight or BMI across the 4 Areas (p>0.05).

Anthropometric Variables 4 year olds	**Area 1 (Urban, Higher F) (n=16) Mean(SD)**	**Area 2 (Rural, Higher F) (n=15) Mean(SD)**	**Area 3 (Urban, Lower F) (n=17) Mean(SD)**	**Area 4 (Rural, Lower F) (n=13) Mean(SD)**	**All areas (n=61) Mean(SD)**	**ANOVA P value[1]**
Height (m)	1.01(0.10)	1.00(0.07)	1.03(0.06)	1.04(0.05)	1.02(0.07)	0.592
Weight (kg)	15.63(2.34)	15.24(2.15)	15.08(1.43)	16.20(1.93)	15.50(1.99)	0.455
BMI (kg/m^2)	15.65(3.72)	15.14(1.85)	14.34(1.30)	14.98(0.94)	15.02(2.27)	0.431

Table 6-7: Mean (SD) height (m), weight (kg) and BMI (kg/m^2) of 4 year olds by area*

*Notes: * Range of drinking water F concentration (ppmF): Area 1: 0 – 1; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6; Range of cooking water F concentration (ppmF): Area 1: 0 – 1; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6; [1] – One way ANOVA*

Table 6.8 shows the nutritional status (stunting and wasting) of 4 year olds by area. Regarding stunting; 45(73.8%) children had normal heights for age while 10(16.4%), 5(8.2%) and 1(1.6%) showed mild, moderate and severe stunting respectively. The distribution was similar for all 4 areas. Wasting was more prevalent in the urban areas - Areas 1 and 3 (31.3% and 35.3% respectively) than in the rural areas - Areas 2 and 4 (20% and 15.4% respectively).

Nutritional status (Waterloo classification) 4 year olds	**Area 1 (Urban, Higher F) (n=16) No.(%)**	**Area 2 (Rural Higher F) (n=15) No.(%)**	**Area 3 (Urban, Lower F) (n=17) No.(%)**	**Area 4 (Rural, Lower F) (n=13) No.(%)**	**All areas (n=61) No.(%)**
Stunting (Height for age)					
Normal	11(68.7)	11(73.3)	13(73.3)	10(76.9)	45(73.8)
Mild	3(18.7)	1(6.7)	3(17.6)	3(23.1)	10(16.4)
Moderate	1(6.3)	3(20.0)	1(5.9)	0(0)	5(8.2)
Severe	1(6.3)	0(0)	0(0)	0(0)	1(1.6)
Wasting (Weight for height)					
Normal	11(68.7)	12(80.0)	11(64.7)	11(84.6)	45(73.8)
Mild	5(31.3)	2(13.3)	6(35.3)	2(15.4)	15(24.6)
Moderate	0(0)	1(6.7)	0(0)	0(0)	1(1.6)

Table 6-8: Nutritional status (stunting and wasting) of 4 year olds by area*.

***Notes**: * Range of drinking water F concentration (ppmF): Area 1: 0 – 1; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6; Range of cooking water F concentration (ppmF): Area 1: 0 – 1; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6*

6.4.5.2 Daily dietary drink (L/day) and food (kg/day) consumption among 4 year olds by area

As Appendix BF shows, the mean (SD) daily drinks consumption for the 4 areas was 1.9(1.3) L/day, with drinking water at 1.7(11.2) L/day consumed in greatest volume while powdered fruit concentrate 0.01(0.02) L/day was consumed in the least volume. Areas 1 and 2 (2.1(2.2) & 2.1(0.8) L/day respectively) accounted for the greatest drinks consumption; it was least in Area 4 (1.4(0.5) L/day) ($p>0.05$) with drinking water consumed in the highest volumes in all 4 areas; from 1.9(2.2) L/day in Area 1 to 1.2(0.4) L/day in Area 4. The mean (SD) daily food consumption per child was 1.5(0.7) kg/day, with cooked rice and beans dishes 0.4(0.2) kg/day representing greatest amount of food consumed while fried/roasted meat at 0.002(0.01) kg/day was the least consumed. In the 4 areas food consumption ranged from a mean of 1.2(0.4) kg/day in Area 1 to 1.8(1.0) kg/day in Area 2 ($p>0.05$). Cereals were not consumed by any participant in Areas 2 and 4.

6.4.5.3 Daily dietary fluoride intake (DDFI) (µg/day) and % contribution from drink and food consumed by 4 year olds by area

The normality of the DDFI data was checked; the Shapiro-Wilk's test was $p<0.05$ and a visual inspection of histograms, normal Q-Q plots and box plots showed that the data were not normally distributed. Therefore the median (minimum, maximum) as well as the mean (SD) were reported.

- **Drinks**

In Appendix BG, the overall mean (SD) DDFI from drinks consumed was 468.8(869.9) µg/day, with the highest F intake (14% of total contribution) being from drinking water (324.0 (757.2) µg/day) while liquid milk/yoghurt provided the lowest contribution to F intake (0.6(1.9) µg/day). The mean (SD) daily dietary F intake from drinks in the 4 areas ranged from 53.4(67.5) µg/day in Area 3 to 1371.3(1356.0) µg/day in Area 2 ($p<0.01$). Drinking water provided the highest mean daily F intake from drinks across the 4 areas ranging from 26.9(59.2) in Area 3 to 1002.0(1287.8) µg/day in Area 2. The overall mean (SD) percentage contribution of all drinks to the TDDFI was 23(23)% (Appendix BG), with drinking water contributing the highest mean (SD)% contribution (14(20)% among the drinks while hot chocolate and carbonated drinks contributed the least (0.1 (0.3)%). The mean (SD) percentage contribution of drinks to total daily dietary fluoride intake ranged from 10(12)% in Area 3 to 33(30)% in Area 1. The highest mean percentage contribution among the drinks to total daily dietary F intake across the 4 areas was from drinking water which ranged from 5(11)% in Area 3 to 18 (26) % in Area 1 ($p<0.01$).

- **Foods**

The overall mean (SD) DDFI from foods (Appendix BG) was 1257.7(1859.5) µg/day, with 493.6(953.4) µg/day and 0.1(0.4) µg/day contributed from soups and fried/roasted meat or meat products respectively. By area, the mean daily dietary F intake from food ranged from 326.5(111.8) µg/day in Area 1 to 3950.6(2111.5) µg/day in Area 2 ($p<0.01$). Soup provided the highest mean F intake at 1786.3 (1230.7) µg/day in Area 2. The overall mean percentage contribution of all foods to the TDDFI was 77(23)% , with cooked rice and beans dishes having the highest mean percentage contribution (22(15)% while raw fruits, cooked/fried fish or seafoods and breakfast cereals contributed the least (1(2)% (Appendix BG). The mean (SD) percentage contribution of all foods to total daily dietary fluoride intake ranged from 67(30)% in Area 1 to 90(12)% in Area 3 ($p<0.01$). Cooked rice and beans dishes contributed most to the total daily dietary F in Areas 1 (22(12)% and 3

(35(17)% while soup (33(21)% and cooked pasta or spaghetti (19 (18) % were main contributors in Area 2 and 4 respectively. Cereals did not contribute to the total daily dietary F intake in Areas 2 and 4 while fried/roasted fish or meat or meat products did not contribute in Area 4.

- **Total daily dietary F intake (TDDFI) from drinks and foods**

The overall mean (SD) TDDFI from drinks and foods consumed was 1726.5(2369.5) µg/day, and ranged from 489.2(145.1) µgF/day in Area 3 to 5321.8(2332.0) µgF/day in Area 2 ($p<0.01$) (Appendix BG).

In summary, and when considered on a daily intake and body weight basis, the overall mean (SD) fluoride intake from drinks was 0.47(0.87) mg/day or 0.03(0.06) mg/kg bw/day while their % contribution to TDDFI was 23(23)% (Table 6.9). The F intake from drinks ranged from 0.05(0.07) mg/day or 0.003(0.004) mg/kg bw/day in Area 3 to 1.37(1.36) mg/day (0.09(0.10) mg/kg bw/day) in Area 2 ($p<0.01$). In contrast, foods represented 77% of the TDDFI. The overall mean (SD) fluoride intake from foods was 1.26(1.86) mg/day (0.08(0.13) mg/kg bw/day) ranging from 0.33(0.11) mg/day (0.02(0.01) mg/kg bw/day) in Area 1 to 3.95(2.11) mg/day (0.27(0.16) mg/kg bw/day) in Area 2 ($p<0.01$). As a result, the mean (SD) overall TDDFI from drinks and foods was 1.73(2.37) mg/day (0.12(0.17) mg/kg bw/day), ranging from 0.49(0.15) mg/day (0.03(0.01) mg/kg bw/day) in Area 3 to 5.32(2.33) (0.36(0.18) mg/kg bw/day) in Area 2. The median (minimum, maximum) of the daily dietary F intake (µg/day) and % contribution from drink and food consumed by 4 year olds by area is shown in Appendices BH and BI respectively.

6.4.5.4 Brand name and type of toothpaste used

The majority 49 (80.3%) of the 4 year olds used Close up toothpaste which contains sodium fluoride (NaF) (Table 6.10). Fifty eight (95.2%) participants used toothpaste that contained sodium fluoride (NaF) while 3 (4.8%) used sodium monofluorophosphate (SMFP) containing toothpaste.

Dietary sources	Area 1 (Urban, Higher F) (n=16)		Area 2 (Rural, Higher F) (n=15)		Area 3 (Urban, Lower F) (n=17)		Area 4 (Rural, Lower F) (n=13)		All areas (n=61)	
	Mean(SD)	Median (Min., Max.)	Mean(SD)	Median (Min., Max.)	Mean(SD)	Median (Min., Max.)	Mean(SD)	Median (Min., Max.)	Mean(SD)	Median (Min., Max.)
Drinks[1]										
mg/day	0.31(0.40)	0.07(0, 1.03)	1.37(1.36)	1.01(0.01, 5.13)	0.05(0.07)	0.02(0, 0.22)	0.17(0.20)	0.11(0.01, 0.68)	0.47(0.87)	0.12(0, 5.13)
mg/kg bw/day	0.02(0.02)	0.01(0, 0.07)	0.09(0.10)	0.05(0, 0.36)	0.003(0.004)	0.002(0, 0.01)	0.01(0.01)	0.01(0, 0.04)	0.03(0.06)	0.01(0, 0.36)
Drinks[1]										
% of total	33(30)	26(0, 81)	24(20)	17(1, 72)	10(12)	6(1, 36)	25(21)	17(4, 71)	23(23)	15(0, 81)
Foods[1]										
mg/day	0.33(0.11)	0.33(0.14, 0.58)	3.95(2.11)	3.72(1.11, 9.52)	0.44(0.14)	0.42(0.26, 0.80)	0.37(0.13)	0.37(0.80, 0.19)	1.26(1.86)	0.41(0.14, 9.52)
mg/kg bw/day	0.02(0.01)	0.02(0.01, 0.04)	0.27(0.16)	0.23(0.07, 0.68)	0.03(0.01)	0.03(0.02, 0.07)	0.02(0.02)	0.02(0.01, 0.04)	0.08(0.13)	0.03(0.01, 0.68)
Foods[1]										
% of total	67(30)	74(19, 100)	76(20)	83(29, 99)	90(12)	94(64, 99)	75(21)	84(30, 96)	77(23)	85(19, 100)
Total dietary F intake[1]										
mg/day	0.63(0.41)	0.48(0.22, 1.36)	5.32(2.33)	4.80(1.12, 10.53)	0.49(0.15)	0.44(0.30, 0.82)	0.54(0.23)	0.51(0.20, 0.96)	1.73(2.37)	0.60(0.20,10.53
mg/kg bw/day	0.04(0.03)	0.03(0.01, 0.09)	0.36(0.18)	0.33(0.07, 0.75)	0.03(0.01)	0.03(0.02, 0.07)	0.03(0.01)	0.03(0.01, 0.05)	0.12(0.17)	0.04(0.01, 0.75)

Table 6-9: Mean (SD) - median (minimum, maximum) daily dietary fluoride intake and mean percentage contribution to daily dietary fluoride intake (mg/day) and (mg/kg bw/day) from drinks and foods consumed by 4 year olds by area*.

Notes: ** Range of drinking water F concentration (ppmF): Area 1: 0 – 1; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6; [1] – One way ANOVA p<0.01 across areas*

Range of cooking water F concentration (ppmF): Area 1:0 – 1; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6.

Toothpaste		No.	%
Brand name	**Fluoride form**		
Close up	NaF	49	80.3
Olive	NaF	5	8.2
Macclean	NaF	3	4.9
Promise	NaF	1	1.6
Maxam	SMFP	1	1.6
Darbul	SMFP	1	1.6
Mymy	SMFP	1	1.6
Total		61	100.0

Table 6-10: Brand name and type of toothpaste used by 4 year olds providing this information (n=61).

6.4.5.5 Estimated fluoride intake from toothpaste

Table 6.11 shows the tooth brushing characteristics and estimated mean (SD) fluoride intake from tooth brushing among 4 year olds by area. The mean (SD) number of brushings per day was 1.23 (0.42) and ranged from 1.39(0.51) in Area 4 to 1.06(0.24) in Area 3 ($p=0.20$). The mean (SD) amount of toothpaste used per tooth brushing in Area 3 was 0.46 (0.29) g while it was 0.63(0.27) g in Area 4 ($p=0.36$), with between 0.82(0.34) mgF (Area 4) and 0.58 (0.37) mgF (Area 3) dispensed per brushing ($p=0.31$). Based on the age of the children studied and estimated as described in section 6.3.14.1, the mean (SD) daily F intake from toothpaste ingestion was estimated to be 0.34(0.23) mg/day (0.02(0.01) mg/kg bw/day), ranging from 0.26(0.16) mg/day (0.02(0.01) mg/kg bw/day) in Area 3 to 0.48(0.28) mg/day (0.03(0.02) mg/kg bw/day) in Area 4 ($p>0.05$).

6.4.5.6 Estimated Total Daily F Intake (TDFI) from diet and toothpaste ingestion

As Table 6.12 shows the overall mean (SD) TDFI among 4 year olds was 2.06(2.40) mg/day (0.14(0.17) mg/kg bw/day), ranging from 0.75(0.24) mg/day (0.05(0.02) mg/kg bw/day) in Area 3 to 5.66(2.38) mg/day (0.39(0.19) mg/kg bw/day) in Area 2 ($p<0.01$). The overall mean (SD) percentage contribution of drinks, foods and toothpaste to TDFI was 17(19)%, 54(21)% and 29(20)% respectively. Drinks made the greatest contribution to TDFI in Area 1 (25(25)%) and the lowest in Area 3 (7(7)%) ($p=0.02$). The mean (SD) % contribution of foods to TDFI was highest in Area 2 at 70(18)% and lowest in Area 4 (40(15)%) ($p<0.001$). There was also a statistically significant lower % contribution of toothpaste to TDFI in Area 2 (Mean (SD) 7(11)%) compared with other Areas ($p<0.01$).

Fluoride intake from tooth brushing	Area 1 (Urban, Higher F) (n=16)	Area 2 (Rural, Higher F) (n=15)	Area 3 (Urban, Lower F) (n=17)	Area 4 (Rural, Lower F) (n=13)	All Areas (n=61)	P value
Mean (SD) No. of brushing per day	1.25(0.45)	1.27(0.46)	1.06(0.24)	1.39(0.51)	1.23(0.42)	0.20
Mean (SD) amount of toothpaste used per brushing (g)	0.49(0.27)	0.53(0.27)	0.46(0.29)	0.63(0.27)	0.52(0.28)	0.36
Mean (SD) F dispensed on toothbrush per brushing (mg/brushing)	0.60(0.38)	0.70(0.35)	0.58(0.37)	0.82(0.34)	0.67(0.36)	0.31
F intake per brushing Mean(SD) (mg/day) Mean(SD) (mg/kg bw/day)	 0.30(0.21) 0.02(0.01)	 0.34(0.23) 0.02(0.01)	 0.26(0.16) 0.02(0.01)	 0.48(0.28) 0.03(0.02)	 0.34(0.23) 0.02(0.01)	 0.05 0.06

Table 6-11: Tooth brushing characteristics and estimated mean (SD) fluoride intake (mg or mg/kg bw/day) from tooth brushing among 4 year olds by area*.

Notes: ** Range of drinking water F concentration (ppmF): Area 1: 0 – 1; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6*

Range of cooking water F concentration (ppmF): Area 1:0 – 1; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6

Fluoride intake	Area 1 (Urban, Higher F) (n=16)	Area 2 (Rural, Higher F) (n=15)	Area 3 (Urban, Lower F) (n=17)	Area 4 (Rural, Lower F) (n=13)	All Areas (n=61)	ANOVA p value	Tukey Post-Hoc
	Mean(SD)	Mean(SD)	Mean(SD)	Mean(SD)	Mean(SD)		
Drinks							
mg/day	0.31(0.40)	1.37(1.36)	0.05(0.07)	0.17(0.20)	0.47(0.87)	<0.01	1v2(p=0.001); 2v3(p<0.01);
mg/kg bw/day	0.02(0.02)	0.09(0.10)	0.003(0.004)	0.01(0.01)	0.03(0.06)	<0.01	2v4(p=0.001); 1v3(p=0.02)
% TDFI	25(25)	22(19)	7(7)	15(14)	17(19)	0.02	
Food							
mg/day	0.33(0.11)	3.95(2.11)	0.44(0.14)	0.37(0.13)	1.26(1.86)	<0.01	1v2(p<0.01); 2v3(p<0.01);
mg/kg bw/day	0.02(0.01)	0.27(0.16)	0.03(0.01)	0.02(0.02)	0.08(0.13)	<0.01	2v4(p<0.01); 1v3(p=0.01);
% TDFI	42(18)	70(18)	62(18)	40(15)	54(21)	<0.01	3v4(p=0.01)
Total diet							
mg/day	0.63(0.41)	5.32(2.33)	0.49(0.15)	0.54(0.23)	1.73(2.37)	<0.01	1v2(p<0.01); 2v3(p<0.01);
mg/kg bw/day	0.04(0.03)	0.36(0.18)	0.03(0.01)	0.03(0.01)	0.12(0.17)	<0.01	2v4(p<0.01)
% TDFI	67(17)	93(6)	68(15)	55(16)	71(20)	<0.01	
Toothpaste							
mg/day	0.30(0.21)	0.34(0.23)	0.26(0.16)	0.48(0.28)	0.34(0.23)	0.05	1v2(p<0.01); 2v3(p<0.01);
mg/kg bw/day	0.02(0.01)	0.02(0.01)	0.02(0.01)	0.03(0.02)	0.02(0.01)	0.06	2v4(p<0.01)
% TDFI	33(17)	7(11)	32(15)	45(16)	29(20)	<0.01	
Total daily F intake							
mg/day	0.93(0.48)	5.66(2.38)	0.75(0.24)	1.02(0.41)	2.06(2.40)	<0.01	1v2(p<0.01); 2v3(p<0.01);
mg/kg bw/day	0.06(0.03)	0.39(0.19)	0.05(0.02)	0.06(0.02)	0.14(0.17)	<0.01	2v4(p<0.01)
% TDFI	100	100	100	100	100		

Table 6-12: Estimated Mean (SD) and percentage contribution to Total Daily Fluoride intake (in mg/day and in mg/kg bw/day) of drinks, foods and toothpaste among 4 year olds by area*.

Notes: ** Range of drinking water F concentration (ppmF): Area 1: 0 – 1; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6*
Range of cooking water F concentration (ppmF): Area 1:0 – 1; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6

6.4.5.7 Urinary fluoride excretion (UFE) characteristics

The mean (SD) urine volume (ml/day) of the 4 year olds was 394(196) ml/day, and ranged from 351(190) ml/day in Area 2 to 462(196) ml/day in Area 4 (p=0.52) (Table 6.13). The overall mean (SD) corrected urine volume was 394(196) ml/24h, and ranged from 353(188) in Area 2 to 459(193) ml/24h in Area 4 (p=0.55). Urinary flow rate was highest (19 (8) ml/h) in Area 4 and lowest 15(8) ml/h in Area 2 (p=0.55). The overall mean (SD) corrected UFE was 0.50(0.42) mg/day (0.03(0.03) mg/kg bw/day), and ranged from 0.34(0.19) mg/day or 0.02(0.01) mg/kg bw/day in Area 4 to 0.83(0.51) mg/day or 0.05(0.03) mg/kg bw/day in Area 2 (p=0.01). A Table 6.14 shows, the overall mean (SD) fractional urinary F excretion (FUFE) for all 61 four year olds was 44(45)%; 18(13)% in Area 2 and 62(52)% in Area 1 (p=0.03).

6.4.5.8 Fluoride retention characteristics

Table 6.14 shows that the overall mean (SD) total daily F retention for the 4 year olds by area was 1.35(2.07) mg/day or 0.09(0.15) mg/kg bw/day, and ranged from 0.29(0.42) mg/day (0.02(0.03) mg/kg bw/day) in Area 3 to 4.28(2.35) mg/day (0.29(0.18) mg/kg bw/day) in Area 2 (p<0.01). The overall daily fractional F retention was 46(45)%, ranging from 72(13)% in Area 2 to 28(52)% in Area 1 (p=0.03).

6.4.5.9 Relationship between fluoride intake and excretion parameters

The correlation between Total Daily F Intake from diet and tooth brushing (mg/day) and:

i. Urinary F Excretion (mg/day): was linear, moderate, positive and statistically significant (ρ=0.41; p=0.001) (Figure 6.2).
ii. Fractional F Retention (%): was linear, moderate, positive and statistically significant (ρ=0.56; p<0.001) at a TDFI lower than approximately 2.5 mgF/day (Figure 6.3). For TDFI values higher than approximately 2.5 mgF/day, the estimated FFR tends to reach limiting constant values independently of how high the TDFI is.

Urinary F excretion characteristics	**Area 1 (Urban, Higher F) (n=16) Mean(SD)**	**Area 2 (Rural Higher F) (n=15) Mean(SD)**	**Area 3 (Urban, Lower F) (n=17) Mean(SD)**	**Area 4 (Rural, Lower F) (n=13) Mean(SD)**	**All Areas (n=61) Mean(SD)**	**ANOVA p value**	**Tukey Post-Hoc**
Urine volume (ml/day)	384(221)	351(190)	386(184)	462(196)	394(196)	0.52	
Corrected urine volume (ml/24h)	384(220)	353(188)	388(185)	459(193)	394(195)	0.55	
Urinary flow rate (ml/h)	16(9)	15(8)	16(8)	19(8)	16(8)	0.55	
Urinary F concentration (µg/ml)	1.30(0.92)	2.56(1.51)	0.97(0.71)	0.81(0.44)	1.41(1.18)	0.00	1v2(p=0.004); 2v3(p<0.01); 2v4 (p<0.01)
Corrected UFE (mg/day) (mg/kg bw/day)	0.46(0.38) 0.03(0.03)	0.83(0.51) 0.05(0.03)	0.38(0.39) 0.03(0.03)	0.34(0.19) 0.02(0.01)	0.50(0.42) 0.03(0.03)	0.01 0.01	2v3(p=0.01); 2v4(p=0.01)

Table 6-13: Mean SD) urinary fluoride excretion characteristics for 61 four-year olds by area*.

Notes: ** Range of drinking water F concentration (ppmF): Area 1: 0 – 1; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6*

Range of cooking water F concentration (ppmF): Area 1:0 – 1; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6

Fluoride retention characteristics	Area 1 (Urban, Higher F) (n=16) Mean(SD)	Area 2 (Rural, Higher F) (n=15) Mean(SD)	Area 3 (Urban, Lower F) (n=17) Mean (SD)	Area 4 (Rural, Lower F) (n=13) Mean (SD)	All Areas (n=61) Mean (SD)	ANOVA p value	Tukey Post hoc
TDFI (mg/day) (mg/kg bw/day)	 0.93(0.48) 0.06(0.03)	 5.66(2.38) 0.39(0.19)	 0.75(0.24) 0.05(0.02)	 1.02(0.41) 0.06(0.02)	 2.06(2.40) 0.14(0.17)	 <0.01 <0.01	1v2(p<0.01); 2v3(p<0.01); 2v4(p<0.01)
UFE(mg/day) (mg/kg bw/day)	0.46(0.38) 0.03(0.03)	0.83(0.51) 0.05(0.03)	0.38(0.39) 0.03(0.03)	0.34(0.19) 0.02(0.01)	0.50(0.42) 0.03(0.03)	0.01 0.01	2v3(p=0.01); 2v4(p=0.01)
TDFR (mg F/day) (mg/kg bw/day)	 0.37(0.58) 0.02(0.02)	 4.28(2.35) 0.29 0.18)	 0.29(0.42) 0.02(0.03)	 0.57(0.37) 0.04(0.02)	 1.35(2.07) 0.09(0.15)	 <0.01 <0.01	1v2(p<0.001); 2v3(p<0.001); 2v4(p<0.001)
FUFE (%)	62(52)	18(13)	54(58)	37(20)	44(45)	0.03	1v2(p=0.03)
Daily fractional F retention (mg/day)%	28(52)	72(13)	36(58)	53(21)	46(45)	0.03	1v2(p=0.03)

Table 6-14: Estimated mean (SD) Total Daily Fluoride Intake (TDFI),Urinary Fluoride Excretion (UFE), Total daily fluoride retention (TDFR) (in mg F/day, mg F/kg body weight/day) and fractional urinary fluoride excretion (FUFE) (%) among 4 year olds by area* (n=61).

Notes: ** Range of drinking water F concentration (ppmF): Area 1: 0 – 1; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6*
Range of cooking water F concentration (ppmF): Area 1:0 – 1; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6

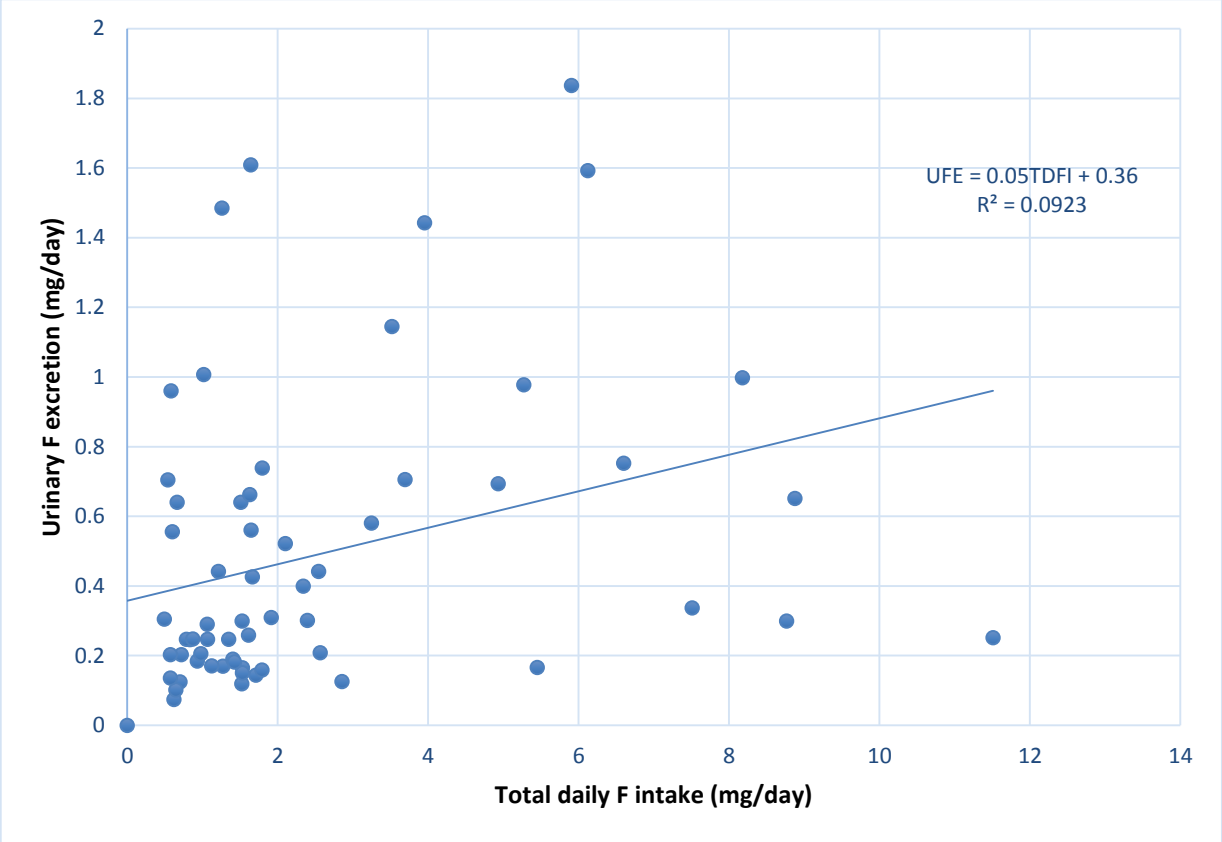

Figure 6-2: Pearsons correlation between Total Daily Fluoride Intake (TDFI) (mg/day) and Urinary Fluoride Excretion (UFE) (mg/day) for 4 year olds (n=61).

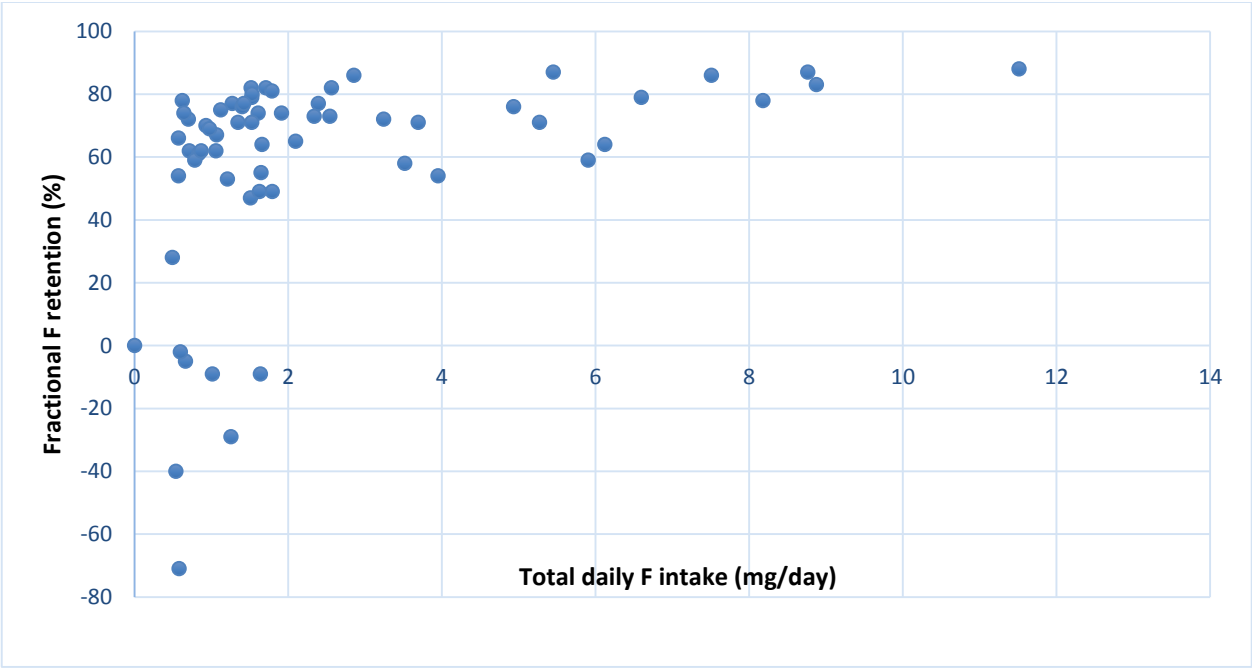

Figure 6-3: Pearsons correlation between Total Daily Fluoride Intake (TDFI) (mg/day) and Fractional Fluoride Retention (FFR) (%) for 4 year olds (n=61).

6.4.5.10 Relationship between nutritional status and F exposure parameters

The mean rank of TDFI, UFE and TDFR was lower among stunted 4 year olds than those with normal % Height for Age but this difference was not statistically significant ($p>0.05$) (Table 6.15). Similarly, the mean rank of these variables was lower among wasted 4 year olds than those with normal % Weight for Age but this difference was not statistically significant ($p>0.05$) (Table 6.16).

F exposure parameters	Stunting (% Height for Age)				
	Normal (n=44)		Undernourished (n=17)		p value
	Mean Rank	Median (min., max.)	Mean Rank	Median (min., max.)	
TDFI	32.18	1.58 (0.54, 11.51)	27.94	1.40 (0.49, 8.76)	0.40
UFE	33.07	0.43 (0.07, 1.84)	25.65	0.26 (0.13, 0.96)	0.14
TDFR	31.36	1.09 (-0.36, 10.11)	30.06	0.96 (-0.42, 7.59)	0.80

Table 6-15: Relationship between stunting and F intake and excretion parameters among 4 years old.

F exposure parameters	Wasting (% Weight for Age)				
	Normal (n=44)		Malnourished (n=17)		p value
	Mean Rank	Median (min., max.)	Mean Rank	Median (min., max.)	
TDFI	32.07	1.57 (0.49, 8.8)	28.24	1.42 (0.58, 11.51)	0.45
UFE	32.70	0.32 (0.07, 1.84)	26.59	0.25 (0.10, 1.61)	0.23
TDFR	32.16	1.13 (-0.42, 7.59)	28.00	1.06 (-0.14, 10.11)	0.41

Table 6-16: Relationship between wasting and F intake and excretion parameters among 4 year olds.

6.4.6 Fluoride exposure - Eight year old study participants

6.4.6.1 Anthropometric and nutritional data

Table 6.17 presents the mean (SD) height, weight and BMI of 8 year olds by area. The difference in mean (SD) of height and weight of 8 year old study participants across the 4 areas were not statistically significant ($p>0.05$) although there was a statistically significant difference in mean BMI ($p<0.05$). The mean (SD) BMI ranged between 14.16(1.27) kg/m^2 in Area 1 and 15.24(1.10) kg/m^2 in Area 4. The nutritional status (stunting and wasting) of 8 year olds showed that overall 41(64.1%) were stunted while 44(68.7%) were wasted (Table 6.21), with 14(70%) of the participants in Area 4 and 8(57.1%) in Area 2 being stunted. Regarding wasting, 11(55%) of the 8y olds in Area 4 while 10 (79.9%) in Area 1 were wasted with 1 (1.7%) child in Area 2 being severely malnourished. Wasting was more prevalent in the urban areas – Areas 1 and 3 (76.9% and 82.4% respectively) than in rural areas – Areas 2 and 4 (64.3% and 55% respectively).

Anthropometric Variables	Area 1 (Urban, Higher F) (n=13) Mean(SD)	Area 2 (Rural, Higher F) (n=14) Mean(SD)	Area 3 (Urban, Lower F) (n=17) Mean(SD)	Area 4 (Rural, Lower F) (n=20) Mean(SD)	All areas (n=64) Mean(SD)	ANOVA p value
Height (m)	1.24(0.08)	1.22(0.09)	1.24(0.04)	1.23(0.07)	1.23(0.07)	0.877
Weight (kg)	21.73(3.35)	21.16(3.67)	22.75(2.51)	23.10(3.04)	22.31(3.15)	0.278
BMI (kg/m^2)	14.16(1.27)	14.19(1.52)	14.84(1.11)	15.24(1.10)	14.68(1.29)	0.041

Table 6-17: Mean (SD) height (m), weight (kg) and BMI (kg/m^2) of 8 year olds by area*

*Notes: * Range of drinking water F concentration (ppmF): Area 1: 0 – 1; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 - 0.6*
Range of cooking water F concentration (ppmF): Area 1:0 – 1; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6

Nutritional status	Area 1 (Urban, Higher F) (n=13) No.(%)	Area 2 (Rural, Higher F) (n=14) No.(%)	Area 3 (Urban, Lower F) (n=17) No.(%)	Area 4 (Rural, Lower F) (n=20) No.(%)	All areas (n=64) No.(%)
Stunting (Height for age) (Waterloo classification)					
Normal	5(38.5)	6 (42.9)	6(35.3)	6(30.0)	23(35.9)
Mild	6(46.1)	3 (21.4)	11(64.7)	12(60.0)	32(50.0)
Moderate	2(15.4)	5 (35.7)	0(0)	2(10.0)	9(14.1)
Wasting (Weight for age) Gomez classification					
Normal	3(23.1)	5(35.7)	3(17.6)	9(45.0)	20(31.3)
Grade 1: Mild malnutrition	6(46.1)	4(28.6)	12(70.6)	10(50.0)	32(50.0)
Grade 2: Moderate malnutrition	4(30.8)	4(28.6)	2(11.8)	1(5.0)	11(17.2)
Grade 3: Severe malnutrition	0(0)	1(7.1)	0(0)	0(0)	1(1.6)

Table 6-18: Nutritional status (stunting and wasting) of 8 year olds by area*.

*Notes: * Range of drinking water F concentration (ppmF): Area 1: 0 – 1; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6;*
Range of cooking water F concentration (ppmF): Area 1:0 – 1; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6

6.4.6.2 Daily dietary food and drink consumption (L/day) and kg/day) among 8 year olds by area

Appendix BJ shows that the mean (SD) daily drinks consumption for the 4 areas was 2.4(1.7) L/day, with drinking water (2.1(1.5) L/day being the drink consumed in greatest volumes while powdered milk (0.01(0.01) litres/day was consumed in the least volume. Area 1 (3.8(2.8) L/day) accounted for the greatest drinks consumption; it was least in Area 3 (1.9(1.1) L/day) ($p>0.01$) with drinking water consumed in the highest volumes in all 4 areas, from 1.6(0.5) L/day in Area 4 to 3.5(2.7) L/day in Area 1. The mean (SD) daily consumption of drinking water was highest in Area 1 (3.5(2.7) L/day) and lowest in Area 4(1.6 (0.5) L/day). The mean (SD) daily food consumption per child was 1.8(1.1) kg/day, with cooked rice and beans dishes 0.6(0.8) kg/day representing greatest amount of food consumed while fried/roasted meat 0.001(0.01) kg/day was the least consumed. In the 4

areas, food consumption ranged from a mean of 1.8(1.6) kg/day in Area 3 to 1.9 (0.8) kg/day in Area 2 ($p>0.01$). Cooked rice and beans dishes were mostly consumed while fried/roasted meat or meat products were least consumed by the study participants in the 4 areas. Cereals were not consumed by any participant in Areas 3 and 4.

6.4.6.3 Daily dietary fluoride intake (DDFI) (µg/day) and % contribution from drink and food consumed by 8 year olds by area

- ***Drinks***

The overall mean (SD) daily dietary F intake (DDFI) from drinks consumed in Appendix BK was 584.9(1118.0) µg/day, with the highest F intake (18% of total contribution) being from drinking water (389.7(766.0) µg/day) while liquid milk/yoghurt provided the lowest F intake of 0.6 µg/day. The mean (SD) daily dietary F intake from drinks in the 4 areas ranged from 137.1(63.0) µg/day in Area 3 to 1799.1(1904.7) µg/day in Area 2 ($p<0.01$). Drinking water provided the highest mean daily F intake across the 4 areas ranging from (103.2(148.3) µg/day in Area 3 to 1192.3(1279.3) µg/day in Area 2. The overall mean(SD) % contribution of all drinks to TDDFI was 28(24)% (Appendix BK) , with drinking water contributing the highest mean % contribution (18(21)%) among drinks while herbal tea, hot chocolate and carbonated drink contributed the least (1(1)%. The mean (SD) % contribution of drinks to TDDFI ranged from 21(22)% in Area 3 to 37(25)% in Area 4. Drinking water contributed between 12(19)% in Area 1 and 24(25)% in Area 4 to TDDFI while liquid milk/yoghurt, powdered milk, or liquid/powdered fruit concentrate made with drinking water made no contribution in any area.

- ***Foods***

Appendix BK also shows that the overall mean (SD) daily F intake from foods consumed by 8 year olds was 1428.9(2042.0) µg/day, with 485.0(1021.3) µg/day and 0.1(0.2) µg/day contributed from soups and fried/roasted meat or meat products respectively. By area, the mean daily dietary F intake from food ranged from 395.0(186.1) µg/day in Area 4 to 4713.0(2134.0) µg/day in Area 2 ($p<0.01$). Soup provided the highest mean F intake at 1952.9(1440.6) µg/day. The overall mean % contribution of all foods to TDDFI was 72(24)% , with cooked rice and beans dishes having the highest mean % contribution (25(20)%), while cooked meat or fried/roasted meat or meat products and confectioneries did not contribute (Appendix BK). Across areas, food contribution to TDDFI ranged from 63(25) % in Area 4 to 79(22)% in Area 3 ($p<0.01$). Cooked rice and beans dishes contributed most to TDDFI in Area 1 33(15)%, Area 3 41(22)% and Area 4 18(15)% while

soup 32(25)% was main contributor in Area 2. Cooked meat or fried or roasted meat or their products did not contribute to TDDFI in any area while cooked/fried/roasted fish/seafoods cereals and confectioneries did not contribute in Areas 2, 3 and 4.

- ***Total daily dietary F intake (TDDFI) from drinks and foods***

The overall mean (SD) daily dietary fluoride from drinks and foods consumed was 2013.7(2692.2) µg/day and ranged from 679.4(302.8) µg/day in Area 4 to 6512.1(2452.6) µg/day in Area 2 ($p<0.01$) (Appendix BK).

In summary and when considered on a daily intake and body weight basis, the overall mean (SD) DDFI from drinks was 0.58(1.12) mg/day or 0.03(0.05) mg/kg bw/day while their % contribution to TDDFI was 28(24)% (Table 6.19). The F intake from drinks ranged from 0.14(0.16) mg/day (0.01(0.01) mg/kg bw/day) in Area 3 to 1.80(1.90) mg/day (0.08(0.01) mg/kg bw/day) in Area 2 ($p<0.01$). In contrast, foods represented 72(24)% of the TDDFI. The overall mean (SD) DDFI from foods was 1.43(2.04) mg/day (0.07(0.10) mg/kg bw/day) ranging from 0.39(0.19) mg/day (0.02(0.01) mg/kg bw/day) in Area 4 to 4.71(2.13) mg/day (0.23(0.10) mg/kg bw/day) in Area 2 ($p<0.01$). The mean (SD) overall TDDFI from drinks and foods was 2.01(2.69) mg/day (0.10(0.13) mg/kg bw/day) ranging from (0.68(0.30) mg/day or 0.03(0.01) mg/kg bw/day) in Area 4 to (6.51(2.45) mg/day or 0.31(0.12) mg/kg bw/day) in Area 2. The median (minimum, maximum) of the daily dietary F intake (µg/day) and % contribution from drink and food consumed by 8 year olds by area is shown in Appendices BL and BM.

6.4.6.4 Brand name and type of toothpaste used

The majority 48(75.0%) of the 8 year olds used close up toothpaste which contain sodium fluoride (NaF) (Table 6.20). Fifty four (85.9%) participants used a NaF based toothpaste.

Dietary sources	Area 1 (Urban, Higher F) (n=13)		Area 2 (Rural, Higher F) (n=14)		Area 3 (Urban, Lower F) (n=17)		Area 4 (Rural, Lower F) (n=20)		All areas (n=64)	
	Mean(SD)	Median (min., max.)	Mean(SD)	Median (min., max.)	Mean(SD)	Median (min., max.)	Mean(SD)	Median (min., max.)	Mean(SD)	Median (min., max.)
Drinks[1]										
mg/day	0.33(0.48)	0.08(0.01, 1.34)	1.80(1.90)	1.23(0, 6.32)	0.14(0.16)	0.06(0.01, 0.50)	0.28(0.29)	0.17(0.01, 0.97)	0.58(1.12)	0.17(0, 6.32)
mg/kg bw/day	0.02(0.02)	0.00(0, 0.07)	0.08(0.01)	0.05(0, 0.27)	0.01(0.01)	0.00(0, 0.02)	0.01(0.01)	0.01(0, 0.04)	0.03(0.05)	0.01(0, 0.36)
Drinks[1] % of total	27(24)	15(1, 67)	24(23)	22(0, 75)	21(22)	0.4(1, 68)	37(25)	36(1, 75)	28(24)	21(0.02, 75)
Foods[1]										
mg/day	0.54(0.40)	0.42(0.7, 1.44)	4.71(2.13)	4.38(154, 9.65)	0.62(0.67)	0.47(0.08, 3.11)	0.39(0.19)	0.36(0.11, 0.93)	1.43(2.04)	0.48(0.08, 9.65)
mg/kg bw/day	0.02(0.02)	0.02(0.01, 0.07)	0.23(0.10)	0.24(0.07, 0.38)	0.03(0.04)	0.02(0, 0.18)	0.02(0.01)	0.02(0.01, 0.05)	0.07(0.10)	0.02(0, 0.38)
Foods[1] % of total	73(24)	85(33, 99)	76(23)	78(25, 100)	79(22)	90(32, 99)	63(25)	64(25, 99)	72(24)	79(25, 100)
Total dietary F intake[1]										
mg/day	0.86(0.77)	0.42(0.23, 2.57)	6.51(2.45)	6.22(1.58, 10.76)	0.76(0.65)	0.60(0.25, 3.13)	0.68(0.30)	0.66(0.26, 1.40)	2.01(2.69)	0.73(0.23,10.76)
mg/kg bw/day	0.04(0.04)	0.02(0.01, 0.13)	0.31(0.12)	0.29(0.08, 0.52)	0.04(0.04)	0.03(0.01, 0.18)	0.03(0.01)	0.03(0.01-0.06)	0.10(0.13)	0.03(0.01, 0.52)

Table 6-19: Mean (SD) - median (minimum, maximum) daily dietary F intake and percentage contribution to total daily dietary fluoride intake (mg/day) and (mg/kg bw/day) from drinks and foods consumed by 8 year olds by area*.

Notes: * *Range of drinking water F concentration (ppmF): Area 1: 0 – 1; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6;* [1] *– One way ANOVA p<0.01 across areas*
Range of cooking water F concentration (ppmF): Area 1:0 – 1; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6.

Toothpaste		No.	%
Brand name	**Type**		
Close up	NaF	48	75.0
Darbul	SMFP	4	6.3
Olive	NaF	4	6.3
Maxam	SMFP	4	6.3
Promise	NaF	1	1.6
Macclean	NaF	1	1.6
Mymy	SMFP	1	1.6
Nil		1	1.6
Total		64	100.0

Table 6-20: Brand name and type of toothpaste used by 8 year olds providing this information (n=64).

6.4.6.5 Estimated fluoride intake from toothpaste

Table 6.21 shows the tooth brushing characteristics and estimated mean (SD) fluoride intake from tooth brushing among 8 year olds by area. The mean (SD) number of brushings per day was 1.08(0.32) and ranged from 1.21(0.43) in Area 2 to (1.00(0.35) in Area 3 (p=0.31). The mean (SD) amount of toothpaste used per tooth brushing in Area 3 was 0.55(0.34) g while it was 0.59(0.6) g in Area 1 (p=0.98), with between 0.74(0.35) mgF (Area 1) and 0.39 mgF (Area 2) dispensed per brushing. Based on the age of the children studied and estimated as described in section 6.3.14.1, the estimated mean (SD) F intake from toothpaste ingestion during tooth brushing per day was 0.31(0.47) mg/day or 0.01(0.02) mg/kg bw/day, ranging from 0.27(0.16) mg/day or 0.01(0.01) mg/kg bw/day in Area 3 to 0.35(0.27) mg/day or 0.02(0.02) mg/kg bw/day in Area 2 (p>0.05).

Fluoride intake from tooth brushing	Area 1 (Urban, Higher F) (n=13)	Area 2 (Rural, Higher F) (n=14)	Area 3 (Urban, Lower F) (n=17)	Area 4 (Rural, Lower F) (n=20)	All Areas (n=64)	ANOVA p value
Mean(SD) no. of brushings /day	1.08(0.28)	1.21(0.43)	1.00(0.35)	1.05(0.22)	1.08(0.32)	0.31
Mean(SD) Amount of toothpaste used per brushing (g)	0.59(0.26)	0.58(0.23)	0.55(0.34)	0.57(0.27)	0.57(0.27)	0.98
Mean(SD) F dispensed on toothbrush per brushing Mean(SD) (mg)	0.74(0.35)	0.66(0.39)	0.66(0.41)	0.73(0.36)	0.70(0.37)	0.87
F intake per brushing Mean(SD) (mg/day)	0.33(0.20)	0.35(0.27)	0.27(0.16)	0.30(0.15)	0.31(0.19)	0.76
Mean(SD) (mg/kg bw/day)	0.02(0.01)	0.02(0.02)	0.01(0.01)	0.01(0.01)	0.01(0.01)	0.42

Table 6-21: Tooth brushing characteristics and estimated mean (SD) fluoride intake (mg/day or mg/kg bw/day) from tooth brushing among 8 year olds by area.

*Notes: * Range of drinking water F concentration (ppmF): Area 1: 0 – 1; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6; Range of cooking water F concentration (ppmF): Area 1:0 – 1; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6*

6.4.6.6 Estimated mean (SD) Total Daily Fluoride Intake (TDFI) (mg/day) and (mg/kg bw/day) among 8 year olds

As Table 6.22 shows, the overall mean (SD) TDFI among 8 year olds was 2.32(2.73) mg/day or 0.11(0.13) mg/kg bw/day, ranging from 0.99(0.33) mg/day or 0.04(0.02) mg/kg bw/day in Area 4 to 6.86(2.52) mg/day or 0.33(0.12) mg/kg bw/day in Area 2 ($p<0.01$). The overall mean (SD) % contribution of drinks, foods and toothpaste to TDDFI was 21(19)%, 54(22)% and 25(17)% respectively. Drinks made the greatest contribution to TDFI in Area 4 (26(20)%) and lowest in Area 3 (14(13)%) ($p=0.23$). The mean (SD) % contribution of foods to TDFI was highest in Area 2 at (71(20) %) and lowest in Area 4 (42(18)%) ($p<0.01$). Toothpaste contribution to TDFI was highest in Area 1 (33(16) %) and lowest in Area 2 (6(6)%) ($p<0.01$).

Fluoride intake	Area 1 (Urban, Higher F) (n=13)	Area 2 (Rural, Higher F) (n=14)	Area 3 (Urban, Lower F) (n=17)	Area 4 (Rural, Lower F) (n=20)	All Areas (n=64)	ANOVA p value	Tukey Post Hoc
	Mean(SD)	Mean(SD)	Mean(SD)	Mean(SD)	Mean(SD)		
Drink							
mg/day	0.33 (0.48)	1.80 (1.90)	0.14 (0.16)	0.29 (0.30)	0.59 (1.12)	<0.01	1v2(p=0.001);
mg/kg bw/day	0.02 (0.03)	0.08 (0.09)	0.01 (0.01)	0.01 (0.01)	0.03 (0.05)	<0.01	2v3(p<0.01);
% of total daily F intake	20 (19)	23 (22)	14 (13)	26 (20)	21 (19)	0.23	2v4(p<0.01)
Food							
mg/day	0.54 (0.40)	4.71 (2.13)	0.62 (0.67)	0.39 (0.19)	1.43 (2.04)	<0.01	1v2(p=0.01);
mg/kg bw/day	0.02 (0.02)	0.23 (0.10)	0.03 (0.04)	0.02 (0.01)	0.07 (0.10)	<0.01	2v3(p<0.01);
% of total daily F intake	47 (14)	71 (20)	59 (24)	42 (18)	54 (22)	<0.01	2v4(p<0.01)
Total diet							
mg/day	0.86 (0.77)	6.51 (2.49)	0.76 (0.65)	0.68 (0.31)	2.02 (2.69)	<0.01	1v2(p<0.01);
mg/kg bw/day	0.04 (0.04)	0.31 (0.12)	0.04 (0.04)	0.03 (0.01)	0.10 (0.13)	<0.01	2v3(p<0.01);
% of total daily F intake	67 (16)	94 (6)	73 (16)	68 (15)	75 (17)	<0.01	3v4(p<0.01)
Toothpaste							
mg/day	0.33 (0.20)	0.35 (0.67)	0.27 (0.16)	0.30 (0.15)	0.31 (0.19)	0.63	
mg/kg bw/day	0.02 (0.01)	0.02 (0.02)	0.01 (0.01)	0.01 (0.01)	0.01 (0.01)	0.39	
% of total daily F intake	33 (16)	6 (6)	27 (16)	32 (15)	25 (17)	<0.01	
Total daily F intake							
mg/day	1.19 (0.89)	6.86 (2.52)	1.03 (0.65)	0.99 (0.33)	2.32 (2.73)	<0.01	1v2(p<0.01);
mg/kg bw/day	0.06 (0.05)	0.33 (0.12)	0.05 (0.04)	0.04 (0.02)	0.11 (0.13)	<0.01	2v3(p<0.01);
% of total daily F intake	100	100	100	100	100		2v4(p<0.01)

Table 6-22: Estimated Mean (SD) and percentage contribution to Total Daily Fluoride intake (in mg/day and in mg/kg bw/day) of drinks, foods and toothpaste among 8 year olds by area*.

Notes: * *Range of drinking water F concentration (ppmF): Area 1: 0 – 1; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6*
Range of cooking water F concentration (ppmF): Area 1:0 – 1; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6.

6.4.6.7 Urinary fluoride excretion (UFE) characteristics

The mean (SD) urine volume (ml/day) of the 8 year olds was 617(336) ml/day and ranged from 395(135) ml/day in Area 2 to 728(341) ml/day in Area 4 (p=0.03) (Table 6.23). The overall mean (SD) corrected urine volume was 618(336) ml/24h and ranged from 394(135) ml/24h in Area 2 to 725(340) ml/24h in Area 4 (p=0.03). The overall mean (SD) urinary flow rate was 26(14) ml/h; it was highest 30(14) ml/h in Area 4 and lowest 16(6) ml/h in Area 2 (p=0.03). The overall mean (SD) corrected urinary F excretion was 0.55(0.69) mg/day or 0.03(0.03) mg/kg bw/day and ranged from 0.27 (0.17) mg/day or 0.01 (0.01) mg/kg bw/day in Area 4 to 1.22 (1.20) mg/day or 0.06 (0.06) mg/kg bw/day in Area 2 (p<0.01). Table 6.29 shows, the overall mean (SD) fractional urinary F excretion (FUFE) for all 64 eight year olds was 36(30)%; highest (56(35) %) in Area 3 and lowest (20(19) %) in Area 2 (p=0.003).

6.4.6.8 Fluoride retention characteristics

The overall mean (SD) total daily F retention for the 8 year olds by area was 1.54(2.19) mg/day or 0.07(0.l1) mg/kg bw/day and ranged from 0.73(0.81) mg/day or 0.05(0.05) mg/kg bw/day in Area 1 to 5.40 (2.60) mg/day or 0.26 (0.13) mg/kg bw/day in Area 2 (p<0.01) (Table 6.24). The overall daily fractional F retention among the 8 year-olds was 54(29)%, ranging from 35(34)% in Area 3 to 70(19)% in Area 2 (p=0.003).

6.4.6.9 Relationship between fluoride intake and excretion parameters

The correlation between Total Daily F Intake from diet and tooth brushing (mg/day) and:

iii. Urinary F Excretion (mg/day); was linear, moderate, positive and statistically significant (ρ=0.57; p<0.001) (Figure 6.4).

iv. Fractional F Retention (%) was linear, moderate, positive and statistically significant (ρ=0.50; p<0.001) (Figure 6.5) at a TDFI of approximately 3.0 mgF/day. For TDFI values higher than approximately 3.0 mgF/day, the estimated FFR tends to reach limiting constant values independently of how high the TDFI is.

Urinary F excretion characteristics	**Area 1 (Urban, Higher F) (n=13) Mean(SD)**	**Area 2 (Rural, Higher F) (n=14) Mean(SD)**	**Area 3 (Urban, Lower F) (n=17) Mean(SD)**	**Area 4 (Rural, Lower F) (n=20) Mean(SD)**	**All Areas (n=64) Mean(SD)**	**ANOVA p value[1]**	**Post-Hoc Tukey p value**
Urine volume (ml/day)	628(482)	395(135)	660(235)	728(341)	617(336)	0.03	2v4(p=0.02)
Corrected urine volume (ml/24h)	627(481)	394(135)	669(239)	725(340)	618(336)	0.03	2v4(p=0.02)
Urinary flow rate (ml/h)	26(20)	16(6)	28(10)	30(14)	26(14)	0.03	2v4(p=0.02)
Urinary F concentration (µg/ml)	0.78(0.64)	2.93(1.82)	0.74(0.38)	0.44(0.29)	1.13(1.33)	<0.01	1v2(p<0.01); 2v3(p<0.01); 3v4(p<0.01)
Corrected urinary F excretion (mg/day) (mg/kg body weight/day)	 0.35(0.26) 0.02(0.01)	 1.22(1.20) 0.06(0.06)	 0.48(0.28) 0.02(0.01)	 0.27(0.17) 0.01(0.01)	 0.55(0.69) 0.03(0.03)	 <0.01 <0.01	 1v2(p=0.002); 2v3(p=0.006); 2v4(p=<0.01)

Table 6-23: Mean SD) urinary fluoride excretion characteristics for 64 eight-year olds by area*.

Notes: * *Range of drinking water F concentration (ppmF): Area 1: 0 – 1; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6;*
Range of cooking water F concentration (ppmF): Area 1:0 – 1; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6

Fluoride retention characteristics	Area 1 (Urban, Higher F) (n=13) Mean(SD)	Area 2 (Rural, Higher F) (n=14) Mean(SD)	Area 3 (Urban, Lower F) (n=17) Mean(SD)	Area 4 (Rural, Lower F) (n=20) Mean(SD)	All Areas (n=64) Mean(SD)	ANOVA p value	Tukey Post-Hoc
TDFI							1v2(p<0.01);
(mg/day)	1.19(0.89)	6.86(2.52)	1.03(0.65)	0.99(0.33)	2.32(2.73)	<0.01	2v3(p<0.01);
(mg/kg bw/day)	0.08(0.07)	0.33(0.12)	0.05(0.04)	0.04(0.02)	0.11(0.13)	<0.01	2v4(p<0.01)
UFE							1v2(p=0.002);
(mg/day)	0.35(0.26)	1.22(1.20)	0.48(0.28)	0.62(0.33)	0.55(0.69)	<0.01	2v3(p=0.006);
(mg/kg bw/day)	0.03(0.04)	0.06(0.06)	0.02(0.01)	0.01(0.01)	0.03(0.03)	<0.01	2v4(p=<0.01)
TDFR							1v2(p<0.01);
(mg F/day)	0.73(0.81)	5.40(2.60)	0.79(0.64)	1.01(0.44)	1.54(2.19)	<0.01	2v3(p<0.01);
(mg/kg bw/day)	0.05(0.05)	0.26(0.13)	0.04(0.04)	0.05(0.05)	0.07(0.11)	<0.01	2v4(p<0.01)
FUFE (%)	39(33)	20(19)	56(35)	29(19)	36(30)	0.003	2v3(p=0.003)
Daily fractional F retention (mg/day)%	51(33)	70(19)	35(34)	61(20)	54(29)	0.003	2v3(p=0.003)

Table 6-24: Estimated mean(SD) Total daily F Intake (TDFI), Urinary Fluoride Excretion (UFE), Total Daily Fluoride Retention (TDFR) (in mg F/day and mg F/kg body weight/day) and Fractional Urinary Fluoride Excretion (FUFE) (%) among 8 year olds by area* (n=64).

Notes: ** Range of drinking water F concentration (ppmF): Area 1: 0 – 1; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6*
Range of cooking water F concentration (ppmF): Area 1:0 – 1; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6

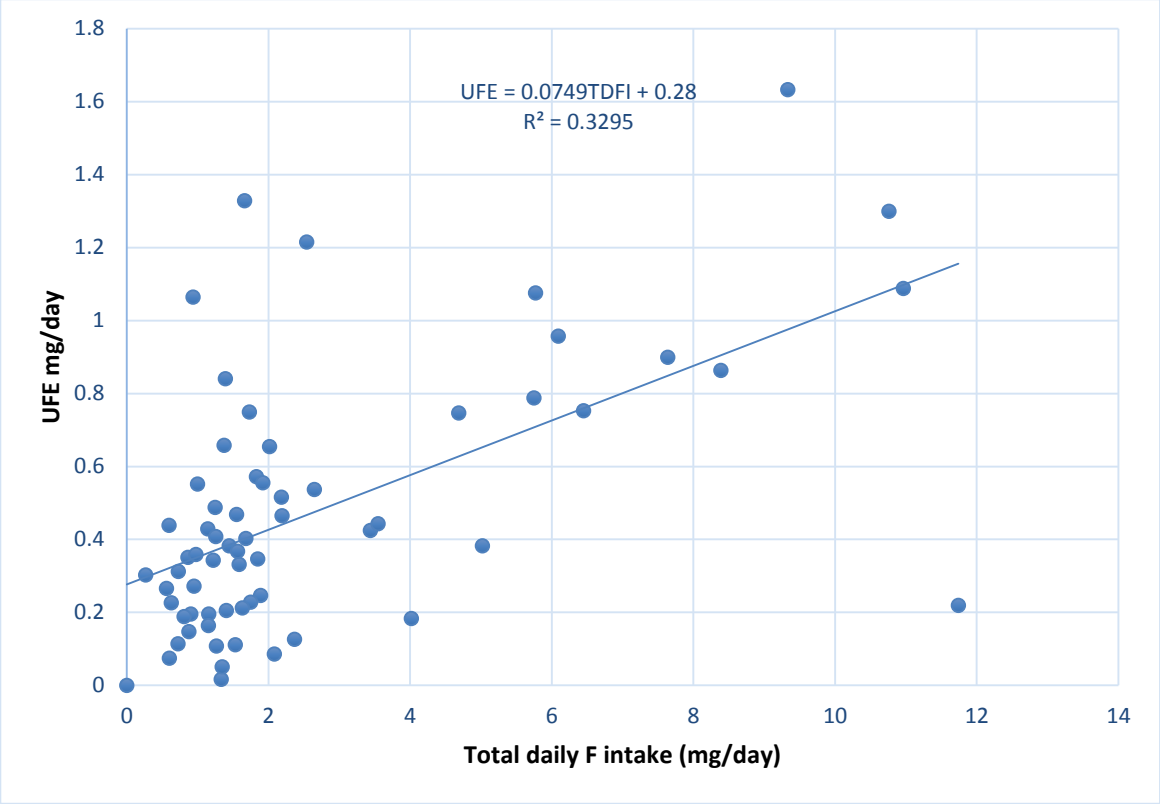

Figure 6-4: Pearsons correlation between Total Daily Fluoride Intake (TDFI) (mg/day) and Urinary Fluoride Excretion (UFE) (mg/day) for 8 year olds (n=64).

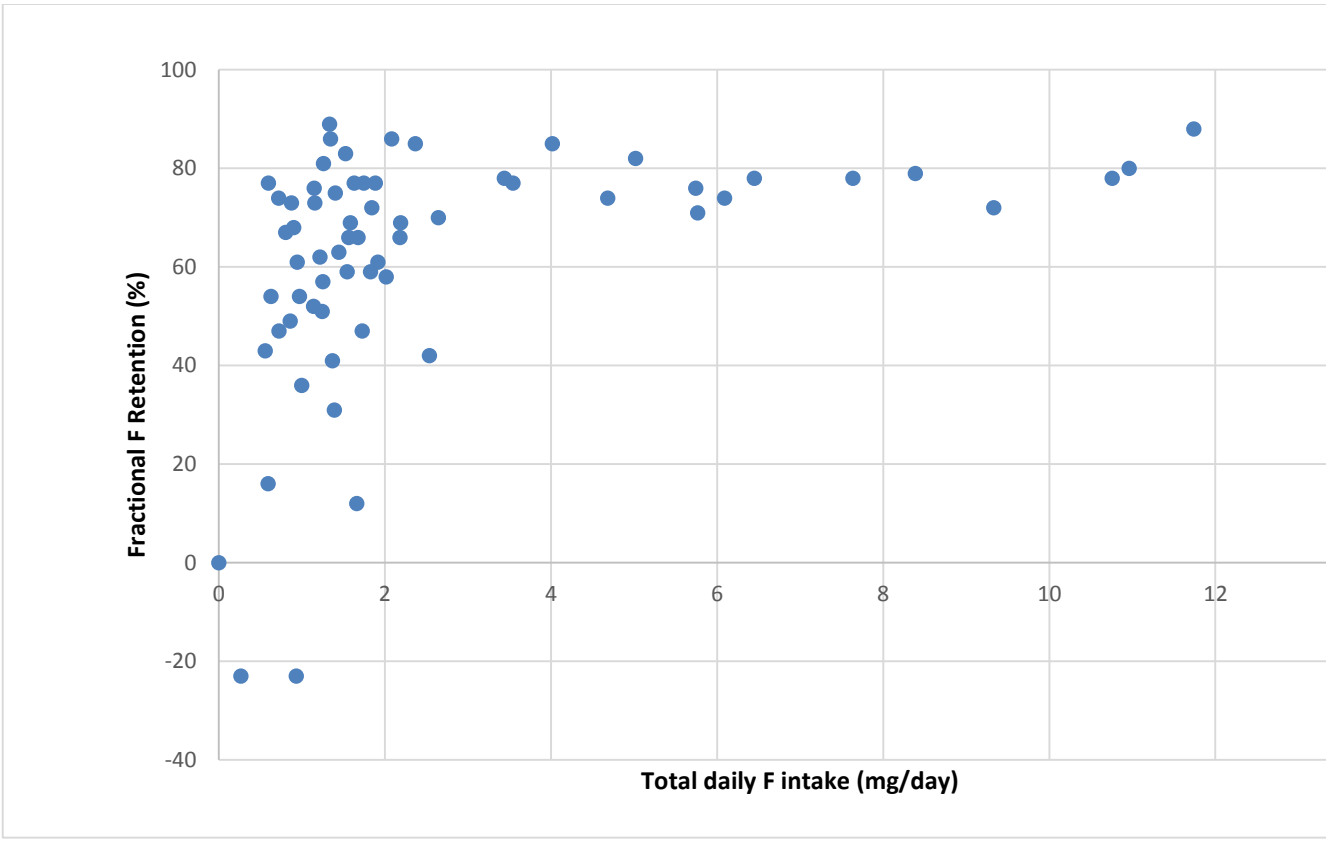

Figure 6-5: Pearsons correlation between Total Daily Fluoride Intake (TDFI) (mg/day) and Fractional Fluoride Retention (FFR) (%) for 8 year olds (n=64).

6.4.6.10 Relationship between nutritional status and F exposure parameters

The mean rank of the TDFI and TDFR was higher among stunted 8 year olds than those with normal height for age but the difference was not statistically significant (p>0.05) (Table 6.25). Conversely, although the mean rank of the TDFI and TDFR was lower among malnourished 8 year olds than those with normal weight for age this difference was also not statistically significant (p>0.05) (Table 6.26).

F exposure parameters	Stunting (% Height for Age)				
	Normal (n=21)		Undernourished (n=43)		p value
	Mean Rank	Median (min., max.)	Mean Rank	Median (min., max.)	
TDFI	30.75	1.40 (0.27, 11.74)	33.55	1.74 (0.59, 10.76)	0.56
UFE	34.08	0.45 (0.02, 5.20)	31.55	0.34 (0.09, 1.30)	0.60
TDFR	29.71	0.99 (-0.06, 10.35)	34.18	1.10 (-0.21, 8.79)	0.35

Table 6-25: Relationship between stunting and F exposure parameters among 8 year olds.

F exposure parameters	Malnutrition (% Weight for Age)				
	Normal (n=21)		Undernourished (n=43)		p value
	Mean Rank	Median (min., max.)	Mean Rank	Median (min., max.)	
TDFI	34.38	1.63 (0.60, 11.74)	31.58	1.58 (0.27, 10.96)	0.57
UFE	29.52	0.36 (0.02, 1.63)	33.95	0.41 (0.11, 5.20)	0.37
TDFR	35.90	1.18 (0.34, 10.35)	30.84	1.15 (-0.21, 8.79)	0.31

Table 6-26: Relationship between wasting and F exposure parameters among 8 year olds.

6.5 Discussion

6.5.1 Design and sample

Estimation of F intake from drinks, foods and toothpaste and F excretion through urine requires skills in epidemiology, sample collection and their laboratory analysis as well as organization. This study was designed to collect drink and food samples consumed, toothpaste samples used and 24 hr urine samples excreted by a subsample of Phase 1 study participants to estimate total F exposure in children. In addition, their heights and weights were measured to determine their nutritional status and explore the effect of this on F exposure and dental defects including dental fluorosis. The study was also designed to estimate the relative contribution of the different components of diet to F exposure in children using a well-designed food frequency questionnaire and separately collecting drink and food samples. In aiming to investigate the relationship between nutritional deficiency

and F intake, excretion and retention, a similar method of assessment to other similar studies was used (Ekstrand et al., 1994a, Ekstrand et al., 1994b, Zohoori et al., 2013a) with both F intake and excretion data collected for each participant. The study was undertaken between the end of January which marks the early part of the dry season and end of June which marks the beginning of the raining season in Nigeria. The atmospheric temperature was not recorded because it was not the aim of the study to investigate the effect of seasonal change on F intake and excretion, however, it is important to mention that water intake varies between and within dry and rainy seasons. This is supported by a previous study (Nyong and Kanaroglou, 1999) on domestic water use in rural semiarid Africa which reported seasonal variation in water consumption. Seasonal variation in the F content of natural water sources has been reported (Larsen et al., 1989).

The samples of 4- and 8-year olds were randomly recruited but were attended to on first come first serve basis rather than alphabetical order or age order from those who participated in Phase 1 of the study in a ratio of 1:8 from private:public schools and were representative of the overall sample in demographic terms. The actual overall recruitment ratio was 8:1 but the numbers completing all aspects of Phase 2 and providing valid samples was 1:6.8 (range 1:3.3 to 1:16 across all 4 areas and 2 age groups). This slight response bias was due to the importance of collecting complete F intake and excretion data which was the reason why the subsample were attended to on first come first recruit approach. This slight response bias does not really matter because the research question was not to compare F intake and excretion between public and private school children. The data for these Phase 2 participants which was collected in Phase 1 on the occurrence of enamel defects, F concentration in drinking and cooking water, health and feeding and tooth cleaning practices during infancy and childhood was used to determine the range and relationship of factors that influence the occurrence of enamel defects, described later in Chapter 8. Including both 4 and 8 year olds in the study provided an interesting and useful opportunity to examine the influence of F intake and retention on the occurrence of developmental enamel defects on both the primary and young permanent dentitions. In addition, at 4 years and above it was feasible to collect urine since these children are usually able to control the urge to urinate and co-operate to ensure complete collection of 24-hour urine. Also at age 4, the primary dentition is usually complete, tooth exfoliation will not have started while children at 8 years have newly erupted permanent incisors and first molars and yet are young enough for data collection on early life feeding habits and behaviours to be more easily recalled by parents/guardians. Additionally, at the age of 8

years, teeth are less likely to have been damaged by caries and erosion making examination for enamel defects and dental fluorosis more straightforward. This sample was also chosen because the findings will inform local, national and international policy decisions on the prevention of developmental enamel defects in both primary and permanent teeth.

6.5.2 Response rate and compliance

Although 128 study participants were planned to be randomly recruited in Phase 2 of the study, 144 parents or legal guardians consented to allow their children or ward to participate. The additional 16 study participants were randomly recruited because their parents pleaded for their inclusion when the sub selection was made after the completion of Phase 1 of the study, because they were keen to find out about the cause of their discoloured teeth. This may have caused selection bias as presumably these children had discoloured teeth but with no effect on the result of the study since comparison was not made between those who had discoloured teeth and those who did not. Of the 144 study participants recruited into Phase 2 of the study 4% (n=6) did not complete the interviewer-administered FFQ because they were called away to attend to some domestic issues, but did not return despite mobile phone reminders. Many parents or legal guardians willingly consented and allowed their child or ward to participate in the study because they were excited and happy to know that the study could help provide information that would help derive strategies in the prevention of enamel defects and their untoward effects on oral and general health related quality of life. Drink and food samples from breakfast, lunch and dinner consumed by study participants were not completely provided by about 6% (n=9) of the study participants because some parents/legal guardians said that they could not afford to buy enough drink or food to provide samples for the study. The non-provision of drink and food samples was worse for the 4-year-olds than the 8-year-olds which could be due to increased financial incapability of parents or legal guardians of the former compared to the latter. The response rate for the 24-hour urine collections was 91.7% which was high when compared to some previous studies and was due to the effective strategy put in place for collection. The parents or legal guardians were reminded to follow urine collection guidelines by reminding them by phone and text messages in the morning before their child went to school, in the afternoon when they returned from school and at night before bed. The urine collection at nurseries and primary schools was also efficient because the teachers closely monitored the study participants. The teachers called the attention of the research assistants to participants who wanted to void urine and they were then closely

monitored to ensure complete collection. Despite the strategy put in place to collect urine at homes, some parents or legal guardians did not fully facilitate urine collection because they were not available at home and their child or ward spent less time at home because they went to neighbouring communities to engage in recreational facilities due to lack of recreational facilities in their homes.

6.5.3 Aim of the study

Obtaining an estimate of F intake and excretion from a stable population of children is required to assess the risk of dental fluorosis since they inform an estimate of F retention or F body burden. Estimate of F intake, excretion and retention as well as other predictors for developmental defects of enamel and dental fluorosis can provide information about aetiological factors of enamel defects. The risk of dental fluorosis might be assessed more easily if it was possible to obtain valid predictions of F intake from measurements of urinary F excretion. Therefore, the aim of the study was to estimate F exposure from a subgroup of the Phase 1 participants by measuring their F intake from drinks, foods and toothpaste, highlighting high F sources and estimating urinary F excretion and retention. The percentage contribution of different components of diet to F intake and the relationship between nutritional status and F exposure were also determined.

6.5.4 Validation and reproducibility of the study

The anthropometric data were collected by one person using standardized measuring devices which were calibrated using a reference height and weight on daily basis before, during and after the data collection thereby ensuring that measurements did not vary. Information about the drink and food consumption pattern was collected using a standardized FFQ (Levy et al., 2013) which was slightly modified to collect information on locally made drinks and foods. The anthropometric and drink and food consumption data were double entered and cross-checked to ensure there were no errors occurring from coding and data entering. The mean difference between the F concentration in drink, food, toothpaste and urine samples when they were first analysed and after re-analysis was very low ranging from 0.004 to 0.033 µg/ml or µg/g. The mean recovery of F added to samples of drink and food before HMDS-diffusion was 94% while it was 96% for F added to toothpaste samples. The recovery recorded in the present study was lower than in previous studies where it ranged between 97% noted by Guha-Chowdhury et al. (1996) to 100.5% recovery reported by Schamschula et al. (1988a) and the reason for these differences might be due to differences in the type of drinks and foods analysed. The mean differences and

mean recovery in this present study indicated satisfactory validity of the methods used in the F analysis. The reliability of the methods for determining F concentration in this study can also be considered adequate since the measurement error was very low. It was important to confirm completeness of 24-hour urine samples due to the challenges associated with collecting these samples; for example, forgetting to collect a urine void could result in underestimation of F excretion. Completeness of 24-hour urine sample collection can be confirmed by using accepted standards for urine flow rate (WHO 2014) and by using external and internal markers. The urine flow rate of study participants was used in this study because it was assumed that it will be difficult to persuade parents to use external markers such as Para-amino-benzoic acid (PABA) tablets for children and children's compliance with tablets. The use of internal markers such as creatinine that is excreted in urine was not practical because of the collection conditions and laboratory costs. A urinary flow rate of 5 to 160 ml/hour and 9 to 300 ml/hour for 4 and 8 year olds respectively was recommended by WHO (WHO, 2014) as adequate. Of the 144 parents who consented to provide 24 hour urine samples 132 (91.7%) provided the samples and 125 (86.8%) urine samples had a urinary flow rate which indicated completeness. This success in obtaining complete 24 hour urine sample or high rate of completeness may be due to the close and frequent contact with parents, at which parents were carefully instructed about the importance of the complete 24-hour collections. Furthermore, parents were interviewed after the collection period to confirm the completeness of the collection and the accuracy of the recorded times.

6.5.5 Anthropometric and nutritional status

The mean height, weight and BMI of 4 year olds was higher than that obtained from 4 year olds Iranian children (Zohouri and Rugg-Gunn, 2000b) but lower than that obtained from their German counterparts (Remer et al., 2002), 4 year olds in the Gaza Strip (Abuhaloob et al., 2015) and 4 year old South-Eastern Nigerian children (Okoroigwe and Okeke, 2009). These differences may be due to differences in genetic characteristics and diets especially the energy and protein intake between these groups of children. In comparison with standards for weight and height (WHO, 2006b), the average weight for height of 4 year old Nigerian children was near to 50^{th} percentile, the average height for age was close to 15^{th} percentile and the average weight for age was near to 25^{th} percentile. The mean BMI for 4 year olds (15.02 kg/m^2) was slightly lower than the WHO standard (15.3 kg/m^2) (WHO, 2006b) and this slight difference could be due to genetic and environmental effects. A study

that quantified genetic and environmental influences on BMI and central adiposity in children showed that the difference between obese and non-obese children could be due to inherited genetic differences (Wardle et al., 2008). BMI in children is also affected by environmental factors such as unhealthy diet, sedentary leisure time and physical inactivity (Lobstein et al., 2004).

In this study, 26.2% of the 4 year olds were stunted and wasted while 64.1% and 68.7% of the 8 year olds were respectively stunted and malnourished. Culturally, in Nigeria, younger children tend to receive better nutritional care than older children and this might be the reason why the nutritional status of 4 year-olds was better than 8 year-olds. The proportion of 4 year olds who were stunted (64.1%) and wasted (68.8%) was higher compared to 7.6% reported among South-eastern Nigerian children (Okoroigwe and Okeke, 2009). The proportion of 8 year olds who were stunted and malnourished was respectively higher than 52.7% and 43% reported for 9-12 year old Nigerian children (Goon et al., 2011) but was lower than 78.5% and 80.1% reported for birth to 12 year old Indian children (Dutta et al., 2009). These differences could be due to differences in socio-economic status of parents. A previous study on socioeconomic status and chronic malnutrition showed an association between socioeconomic status of parents such as wealth and education and stunting (Urke et al., 2011).

6.5.6 Methods used in dietary survey

It is difficult to evaluate the reliability and validity of dietary surveys. However, it has been previously reported that prospective methods of assessing dietary intake such as food recording using dietary diary and interview technique can provide a valid estimation of the mean intake since more detailed qualitative information such as brand name, cooking and preparation methods and time of consumption can be recorded (Hackett et al., 1983). These tools also allow some methods such as the use of the Physical Activity Level (PAL) to be used to confirm validity when dietary diaries are used (Torun, 2005). Period of food recording can vary from 2 to 7 consecutive days but it has been shown that recording for more than 4 consecutive days may result in decrease in reported intakes (Thompson and Byers, 1994). Duplicate diet, a form of prospective method and retrospective methods such as 24-hour recall, market basket collection, diet history and FFQ have been used to assess dietary F intake. As the aim of this present study was to measure current F intake of children, a standardized semi-quantitative FFQ was adapted from the IOWA F study (Levy et al., 2013) and slightly modified to collect information about frequency of consumption

and portion sizes of locally consumed drinks and foods. Using this FFQ and asking respondents to select portion size from household measures made it practical and cost-effective to collect information about food and drinks consumption patterns. The provision of household measures facilitated the recall of portion sizes of drinks and foods consumed. Information about the previous dietary intake would have been relevant because it would have helped to show any historical associations between diet and the occurrence of enamel defects. However, it would have been difficult to assess this information accurately in this present study because of respondent and memory bias and, on balance, the choice of using a FFQ to explore F intake was more appropriate. Previous studies have used food diaries to collect information on dietary F intake from 4 year old Iranian (Zohouri and Rugg-Gunn, 2000b), 6 to 7 years British (Maguire et al., 2007) and 3 to 4 years Palestinian children (Abuhaloob et al., 2015). Other studies have used duplicate diets to collect such information from 4 to 5 year old Colombian (Franco et al., 2005b), 4 to 6 year old Brazilian (Rodrigues et al., 2009) and 6 to 7 year old British (Zohoori et al., 2013b) children. Food diaries could not be used in this present study because it was assumed prior to undertaking the research that the majority of parents or legal guardians, especially mothers, might not be able to read or write. Duplicate diet could also not be used because of the cost of duplicating the diet and the huge burden on participants which might make participants alter dietary habits to ease the burden. The success of the use of a semi-quantitative FFQ has been reported in previous studies on F intake from diets among 3 to 6 year old American children involved in the IOWA F study (Levy et al., 2003), 2 to 6 year old Brazilian (Miziara et al., 2009, Levy et al., 2013) and 1 to 9 year old Saudi Arabian children (Akpata et al., 2014).

6.5.7 Amount of drink and food items consumed

The amount and type of drink and food items consumed can affect the extent of F absorption and excretion and subsequently F balance. Drinks such as milk and foods that contain considerable amounts of divalent or trivalent cations (e.g. calcium) reduce F absorption as a result of the formation of insoluble complexes of these cations with F (Zohoori et al., 2013a). On the contrary, diets high in fat may increase the absorption of F by lowering the gastric emptying time. In addition, diets such as meat-based diets result in a more acidic urine and therefore less urinary F excretion. Vegetarian-based diets result in alkaline urine and therefore more urinary F excretion (Buzalaf and Whitford, 2011). If the urine is acidic, more F ions are converted to HF which is diffusible across tubular

epithelium while if it is alkaline nearly all the F will exist in the ionic form and remain within the tubules to be excreted.

On average, each individual child in this study consumed a higher amount of food (1.67kg/day) compared to those reported in a study of 1-3 year olds in Mexico at 0.56 kg/day (Martínez-Mier et al., 2003) mainly due to differences in age but also geographical location, environmental factors and type of diet. In this present study, cooked rice and beans dishes was the food mostly consumed by study participants probably due to the general preference of Nigerian children for these food items. On average, each Nigerian child in this study consumed 2.33 L/day of drinks, this high consumption of drinks could be due to high loss of body fluids from the hot tropical weather. Water was the drink that is mostly consumed (1.88 L/day) by the study participants because it is easily available from wells and boreholes and it is also sold in cheap sachets. In Nigeria, wells and boreholes were the most common source of drinking water because the pipe-borne water is grossly inadequate especially in rural areas, therefore the majority get their water from ground water. The amount of water consumed by children in different studies was 1.14 L/day for 4 year old Iranian (Zohouri and Rugg-Gunn, 2000a), 1.52 L/day for 4 to 6 years American (Ershow and Cantor, 1989), 1.57 to 2.06 L/day for 8 to 9 year-old Mexican (Grijalva-Haro et al., 2001) and 0.27 L/day to 0.30 L/day for 4 to 6 years Peruvian (Rodrigues et al., 2009) children. The observed differences in the amount of water consumed might be due to differences in weather conditions when the studies were undertaken since warm atmospheric conditions result into increase consumption of water than in cold conditions. The mean volume of beverages (0.21 L/day) consumed in this study was lower than the amount consumed by 2 to 8 year old Japanese (0.60 to 0.71 L/day) (Nohno et al., 2006) and 4 to 6 year old US (1.05 L/day) (Pang et al., 1992) children probably due to differences in economic status of parents, availability of drinks, dietary practices and age group studied. Overall, a true comparison of drink and food consumption studies is difficult due to the differences in the age of children, environmental temperature as well as the differences in dietary habits.

6.5.8 Toothpaste use

Toothpastes containing F are widely used by different population groups worldwide. However, swallowing toothpaste during tooth brushing has been reported as a risk indicator for dental fluorosis in low or non-fluoridated communities (Rugg-Gunn et al., 1998). Studies have reported that frequent tooth brushing and tooth brushing before 2 years old

could increase the occurrence of developing dental fluorosis (Osuji et al., 1988b, Vallejos-Sanchez et al.). In this present study, the F form in the toothpaste was not specified on one label and the F concentrations not specified on 3 labels. This information is required for consumers to make an informed choice on the type of toothpaste that is suitable for use especially for children. More than 85% of the participants used sodium fluoride toothpastes since these type of toothpastes are the most commonly available in the Nigeria market. The mean number of brushings per day was slightly higher in 4 year olds than 8 year olds probably because of the general belief among Nigerians that younger children consume more cariogenic diets than older children and as such they need more stringent oral hygiene measures. However, the mean amount of toothpaste used and dispensed per brushing was higher in the 8 year olds than the 4 year olds possibly due to a general belief in the Nigerian community that the appropriate amount of toothpaste to be used is directly associated with a child's age, but also because these children were less likely to be supervised by adults. The mean amount of toothpaste used by 4 year olds (0.52g) was higher than the pea-size (0.25g) recommended for this age group in Britain (DoH/BASCD, 2009) and America (American Dental Association, 2014). It was also higher than 0.3g recommended for young Brazilian children (Cury and Tenuta, 2014). This greater usage of toothpaste may be attributed to the poor knowledge of parents or legal guardians and their children or wards to adequate use of F toothpaste and unsupervised application of toothpaste to toothbrush during tooth brushing. Previous studies have reported that the mean amount of toothpaste used per brushing was 0.45g for 4 year old Canadian (Naccache et al., 1992), 0.36g for 30-month-old English (Bentley et al., 1999), 0.43g for 4-7-year old Brazilian (Pessan et al., 2003) and 0.49g for 1-3-year old Brazilian (De Almeida et al., 2007) children. The mean amount of toothpaste used in this current study was higher than that reported in a multi-country cross-sectional study (Cochran et al., 2004b) on F ingestion from toothpastes in 7 European countries among 1.5 to 4.6 years children in which the mean amount of toothpaste used per brushing was 0.36g, 0.41g and 0.49g for the age ranges 1.5 to 2.5, 2.5 to 3.5 and greater than 3.5 years respectively. The observed differences in the mean amount of toothpaste used per brushing in various studies might be due to differences in the type of toothpaste used, analytical method used to measure F concentration of the samples, age group studied and local beliefs about tooth brushing.

6.5.9 Concentration of F in drink, food and toothpaste items

- **Collection and transportation of drink and food samples**

Samples of drink and food consumed by study participants during breakfast, lunch and dinner on a single day rather than for 2 or 3 days were collected because the study locations comprised mainly people of low socio-economic groups who might not be able to afford the cost of providing samples for more than 1 day. The research assistants visited homes of study participants to collect breakfast, lunch and dinner samples in the morning, afternoon and evening respectively to ensure adequate collection of these samples. Fluoride concentrations in drink and food samples can be affected by the growth of microbial flora on these items because these organisms absorb F onto their cell walls (Marguis, 1995). Therefore, it was necessary for these samples to be immediately transported after collection using a cold bag containing dry ice to a freezer placed at the Oral Pathology Laboratory, University of Ibadan where the samples were frozen and kept frozen during transportation to, and in, Newcastle, prior to analysis.

- **F concentration in drink and food samples**

Interest in the F content of drinks and foods originates from reports that, concomitant with the dramatic reduction in dental caries in both optimally and negligibly fluoridated communities because of the implementation of water fluoridation (Ripa, 1993, Carstens et al., 1995), there has been an increase in the prevalence of mild-to-moderate dental fluorosis (Pendrys, 1995, Pendrys et al., 1996). Furthermore, the difference in the prevalence of dental fluorosis between optimally fluoridated and negligibly fluoridated communities has narrowed considerably (Clark, 1994, Selwitz et al.) due to increased exposure to F from many sources, including food and drinks. Jackson and colleagues (Jackson et al., 2002) reported in their study on F content of foods and beverages that comprehensive data about F exposure from all sources should be collected in order to explore possible reasons for reported increases in the prevalence of dental fluorosis in any community.

In this study, drinks and foods were categorised into subgroups (Table 6.3) according to their composition and method of preparation, whether they needed water for preparation or prepared at home or by manufacturer. Analysis of FFQ data revealed that overall 14 drink and 17 food groups were the main dietary components reported as consumed by participants. Excluding drinking and cooking water samples, 15 samples of 3 drink groups and 599 samples of 8 food groups were provided for F concentration measurement. Therefore, about 36% of the drink and food groups were available for F analysis but these 3 drink and 8 food groups represented the diets that were commonly consumed by children in these Nigerian settings. Considerable amounts of water are required for the preparation of

certain foods and therefore F in the water can be concentrated in these foods. It is particularly important to know the F content in those drinks and foods commonly consumed by children during the period of increased risk of developing dental fluorosis (de Carvalho et al., 2013).

The widely used direct and indirect (hexamethyldisiloxane diffusion) methods and F-ISE was used in this current study as in other previous studies (Zohouri and Rugg-Gunn, 2000a, Franco et al., 2005b, Maguire et al., 2007, De Almeida et al., 2007) to measure F concentration of collected samples. The F concentration of drinks ranged between 0.013 to 0.487µg/ml and were usually made from drinking or cooking water. Based on the water samples provided by parents or legal guardians the highest F concentration of drinks would have been expected in Area 2 which had the highest F concentration of drinking and cooking water. However, the highest F concentration in drinks (0.487µg/ml) was found in hot chocolate drinks in Area 3 (Urban: mean water F ranging from 0-0.2 ppm) since there was no hot chocolate sample from Area 2. The F concentration of foods varied from 0.05 to 6.117µg/g primarily due to the F concentration of water used to prepare the food items. The highest F concentration in foods was found in soup in Area 2 (Rural: mean water F ranged from 0-4 ppm) confirming the association between F concentration in water and F concentration foods prepared using this water. In addition, it confirmed that water samples provided by parents or legal guardians in this area represented the same water supplies used to prepare the foods.

- **F concentration in toothpaste samples**

In this study, the measured F concentration in only one toothpaste was higher than what was stated on the toothpaste label. For the remaining toothpaste where the measured F concentration was between 73 and 92% of the label values. A previous study that evaluated F stability of toothpastes sold in Manaus in Brazil reported a loss of 40% of total F content. A report by the US Food and Drug Administration stated that the soluble F ions in toothpastes should not be less than 60% of the total F content, therefore, the measured F of the total F content is acceptable (Carrera et al., 2012). A previous study (Cochran et al., 2004b) that analysed 188 toothpaste samples with different batch numbers from seven European countries showed that the F content of 59% of them had lower values when compared to the labelled values while 16% had higher values. These differences may be due to an actual difference in the free ionic F measured, inaccurate labelling of F

concentration of the toothpaste, as well as instability of the soluble F in the toothpaste during storage (Feitas, 1984, de Oliveira Conde et al.).

6.5.10 Dietary fluoride intake

The drink and food samples collected from parents or legal guardians were analysed for F concentration, however, there were a number of foods and drinks for which no samples could be collected. Therefore actual F contents of these samples could not be determined. A UK F database (Maguire et al., 2012) provided the best alternative estimate for F contents of these samples. In addition, the F concentration of individual drinking water collected was used to represent herbal tea, hot drinks, liquid and powdered fruit concentrate made with water. For some foods such as raw vegetables, cooked fish/sea food and meats the UK F database values were used. In future studies, reimbursing parents/legal guardians from this setting or similar setting will ensure that they provide the drink and food samples consumed by children. After F analysis, the F concentration of ready-to-eat breakfast cereal and carbonated drinks collected in Areas 1 and 3 was stated for Areas where these samples were not collected. This was considered appropriate because these ready to eat samples were also sold in all the areas. Food types such as steamed vegetables, fruits and rice or beans dishes and drink types such as sugar-free fruit concentrate and ready-to-consume drink as well as sugared ready-to-consume drink consumed by less than 10 participants were ignored in the estimate of dietary F intake. In addition, the amounts of these drink and food items consumed by the participants were small and were unlikely to have an effect on the estimate of dietary F intake.

The mean dietary F intake from food of 1.26 mg/day among 4 year olds living in naturally fluoridated areas in this current study was higher compared with 0.13mg/day, 0.15 mg/day, 0.35 mg/day and 0.52-0.59 mg/day reported for twelve 15 - 30 month olds American (Martinez-Mier et al., 2009), and fifty-four 16 to 40 month olds American (Rojas-Sanchez et al., 1999), twenty 3-5 year old Chilean (Villa et al., 2000) and forty-six 1.3 to 3 year old Mexican (Martínez-Mier et al., 2003) children respectively. Furthermore, on the basis of body weight, it was 0.08 mg/kg bw/day higher compared to 0.01 – 0.02 mg/kg bw/day, 0.02 mg/kg bw/day, 0.03 mg/kg bw/day and 0.02-0.03 mg/kg bw/day reported for twenty nine less than 4 year old Brazilian (Zohoori et al., 2013a), sixty-six 3 to 4 year-old New Zealand (Guha-Chowdhury et al., 1996) and two hundred and sixteen 3 to 4 year old Palestinian (Abuhaloob et al., 2015) children respectively living in fluoridated areas. The observed differences in dietary F intake seen in these studies are most likely due to

differences in dietary practices and in the method of dietary intake assessment used, but may also be due to the F analysis method used by the researchers.

With regard to F intake from drinks, the mean dietary F intake from drink of 0.47 mg/day or 0.03 mg/kg bw/day for 4 year olds in the present study was higher than intake reported for the US children (0.42 mg/day) (Martinez-Mier et al., 2009) and was four times higher compared with a Mexican population whose domestic salt was fluoridated (200-250 mgF/kg) (Martínez-Mier et al., 2003). It was also higher than F intakes (0.42 mg/day) reported for 1.3 to 3.3 year old US children living in Indianapolis (Rojas-Sanchez et al., 1999) and 3 to 5 year olds in Chile (Villa et al., 2000) and lower than the F intake (1.2 to 8.8 mg/day) reported for thirty, < 5 year olds living in higher natural F areas (1.8-2.1 ppmF) of Ethiopia (Malde et al., 2003). Differences in the sources and levels of F exposure are likely to be the main reasons for the reported differences in the dietary F intake seen in these studies. Adding various concentrations of F in water to children drinks such as powdered milks, formulas and juices can significantly influence the amount of dietary F intake from drinks(Buzalaf et al., 2004).

The mean dietary F intake from food of 1.43 mg/day among 8 year olds living in naturally fluoridated areas of Nigeria found in the present study was higher compared with 2.31 mg/day reported for 20, 8 to 9 year old Mexican children living in fluoridated areas (Grijalva-Haro et al., 2001) probably due to differences in geographical locations, dietary habits and the method of assessment (duplicate plate). When F consumption through drinks was compared, when estimated using a FFQ it was between 0.01 to 0.02 mg/kg bw/day for 1 to 9 year old Kuwaiti children living in fluoridated areas; lower than the 0.03 mg/kg bw/day found in the 8 year old Nigerian children probably because of age group differences and method of assessing dietary intake.

Many studies on dietary F intake from drinks among children have not reported F intake from water and this could possibly be due to the lower water and higher beverage consumption in these populations. However, F intake from drinks has been reported by some researchers (Villa et al., 2000, Martínez-Mier et al., 2003, Akpata et al., 2014) which has accounted for F intake from both water and beverage. In addition, some studies have reported F intake from water only, for example, a study among twenty 8 to 9 year old Mexican children living in an optimally fluoridated area (Grijalva-Haro et al., 2001) reported that F intake from water was 1.61 mg/day which is higher than the 0.39 mgF/day for 8 year olds in this current study. F intake from water for 4 year olds (0.32 mg/day) was

higher compared with 0.18 mg/day and 0.34 mg/day reported for thirty-three 1-3 (De Almeida et al., 2007) and twenty-five 4-6 (Rodrigues et al., 2009) year old Brazilians respectively as well as the 0.04 mg/day and 0.13 mg/day for forty-six Peruvian 4 to 6 year olds who lived in areas where milk and salt are fluoridated (Rodrigues et al., 2009). Some of the differences in the reported F intake from water in various studies might possibly be due to differences in the amount of water consumed. Overall, the F intake from water in this present study was higher than F intake from other drinks because water was the drink most commonly consumed by the study participants. Foods contributed more to the TDDFI than drinks for all children in this study (77% for 4 y olds; 72% for 8 y olds) and this finding is in agreement with Martinez-Mier and colleagues (Martínez-Mier et al., 2003) who reported between 81 to 84% contribution from food to TDDFI among forty-six 1.5 to 3 year olds living in salt fluoridated areas of Mexico. This might be due to the amount and F concentration of water used to prepare the food as well as types of foods consumed. The higher contribution of food to TDDFI in the Mexican study when compared to this Nigerian study might be due to the fluoridised salt used in cooking. On the contrary, some studies (Rojas-Sanchez et al., 1999, De Almeida et al., 2007) reported higher contribution from drinks compared with foods, probably due to difference in dietary habits; i.e. amount, F concentration and types of drinks and foods consumed.

Total daily dietary F intake has been reported by many studies in both fluoridated and non-fluoridated areas ranging from 0.36 mg/day for 3 to 4 year children in New Zealand (Guha-Chowdhury et al., 1996) to 2.31 mg/day for 8 to 9 year old Mexican (Grijalva-Haro et al., 2001) children. This wide range in the TDDFI might be due to variation in dietary habits, geographical locations as well as types and quantities of food and drinks consumed. The mean daily dietary F intake for 4 year olds in this study (1.73 mg/day) was higher compared to other studies undertaken in both fluoridated and non-fluoridated areas except in a cross-sectional study among 1-4 year old Kenyan children living in an area with 9ppmF in the water supply (Opinya et al., 1991b) in whom the TDDFI was 14.5 mg/day and 8 to 9 year old Mexican (Grijalva-Haro et al., 2001) children living in high fluoride areas (2.77ppmF in water) in whom the TDDFI was 2.31 mg/day. These differences could be due to differences in nutritional status of study participants, age group studied and dietary practices as well as the difference in F concentration of the water supply. Fasting child may absorb F from water or other sources more quickly than a well-fed child due to the inexistence of complexes of F in an empty stomach (Buzalaf and Levy, 2011). A

malnourished child may have low F deposition over a long term period of time due to slower bone growth (Buzalaf and Levy, 2011).

6.5.11 Fluoride intake from toothpaste

The estimated mean F intake in this present study was made by pictorial assessment of the amount of toothpaste placed on toothbrush and evidence from other studies (Franzman et al., 2006, Levy et al., 2010) about the amount that this represent. The amount of F ingested from toothpaste during tooth brushing was estimated based on the mean proportion of toothpaste ingestion per tooth brushing session among 4 to 6 year old UK children (Zohoori et al., 2012). This method of assessing F intake from toothpaste is useful in epidemiological studies involving large number of people where individual tooth brushing behaviours cannot be observed, however, it is subject to recall memory bias. The mean F intake from toothpaste was estimated to be 0.34 mg/day and 0.31 mg/day for 4 and 8 year olds respectively probably due to greater number of 4 year olds who inadvertently ingested toothpaste, which is a habit more likely in younger children, compounded by the lower proportion of 4 year olds who did not rinse their mouth after toothpaste use. Also, it shows that despite dispensing more toothpaste on the toothbrush by 8 year olds, their F intake from toothpaste ingestion was less than that of 4 year old. It emphasizes the issue of supervising young children and educating their parents on correct tooth brushing practice. The inverse relationship seen between F intake and age was in agreement with the finding for Canadian children (Osuji et al., 1988a, Naccache et al., 1992). The greater tendency for younger children to swallow more toothpaste is of some concern, particularly in high water fluoride areas due to the additive effect of F in toothpaste and water. The mean F intake from toothpaste for 4 year olds was lower than 0.42, 0.43, and 1.34 mg/day reported for 2.6 year old English (Bentley et al., 1999), 1.3 to 3.3 year old American (Rojas-Sanchez et al., 1999) and 1 to 3 year old Brazilian (De Almeida et al., 2007) children living in fluoridated areas. It was also lower than 0.58 and 1.21 mg/day for 1.3 to 3.3 year old American (Rojas-Sanchez et al., 1999) and 3 to 4 year old New Zealand (Guha-Chowdhury et al., 1996) children living in non-fluoridated areas. Furthermore, it was lower than 0.61 mg/day for 2 to 6 year old Brazilian children who lived in fluoridated areas and whose dietary information was also collected using FFQ (Miziara et al., 2009). However, it was higher than 0.25 mg/day for 3 to 5 year old Chilean (Villa et al., 2000) and 0.26 mg/day for some 3 to 4 New Zealand (Guha-Chowdhury et al., 1996) children living in fluoridated areas. It was also higher than 0.06 mg/day for 4 year old Iranian (Zohouri and Rugg-Gunn, 2000b)

and some 3 to 4 year olds New Zealand (Guha-Chowdhury et al., 1996) children living in non-fluoridated areas. There is scarcity of studies to compare F intake from toothpaste among 8 year olds; most have been undertaken among lower age groups. However, the mean F intake from toothpaste of 0.01mg/kg bw/day found for 8 year olds in this current study was lower than the 0.02 mg/kg bw/day seen for 6 to 7 year old UK children living in fluoridated and non-fluoridated areas (Maguire et al., 2007). The differences in the F intake from toothpaste seen in these studies could be due to differences in tooth cleaning practices, the F concentration of toothpaste used, the age of study participants and method of assessing F intake from toothpaste.

6.5.12 Total daily fluoride intake

In this study, diet and toothpaste were the only sources of F intake in these children, no child took any type of F supplement. The mean total daily F intake was 2.06 mg/day or 0.14 mg/kg bw/day and 2.32 mg/day or 0.11 mg/kg bw/day for 4 and 8 year olds respectively, most likely due to differences in age and dietary practices. For 4 and 8 year-olds, only Area 2, showed a higher mean TDFI (0.39, 0.33 mg/kg bw/day respectively) compared to optimal range of 0.05-0.07 mg/kg bw/day while it ranged between 0.04-0.08 mg/kg bw/day in other areas. This might be the reason for the observed higher prevalence of DDE and dental fluorosis in Area 2 in Chapter 5. On the basis of body weight, the total daily F intake range from 0.01 to 0.02 mg/kg bw/day for less than 4 year old Brazilian (Zohoori et al., 2013a) and 1.3 to 3.3 Mexican (Martínez-Mier et al., 2003) children living in fluoridated areas respectively. It also ranged from 0.03 to 0.07 mg/kg bw/day among 3 to 4 year New Zealand (Guha-Chowdhury et al., 1996), 2 to 3 year old Colombian (Franco et al., 2005a) and 1.3 to 3.3 year old US (Rojas-Sanchez et al., 1999) children living in non-fluoridated areas. Variations in the age group studied seen across all these studies, as well as differences in sources and amount of F exposure might be the reason for the observed differences in total daily F intake. The observed differences could also be from the different F analytical methods employed as well as differences in assessment of F intake from diet and toothpaste.

Differences in geographical locations and tooth cleaning practices might be the reason why the total daily F intake of 4 year olds was higher than 0.02, 0.07, 0.09 and 0.10 mg/kg bw/day for 2 to 6 year old Brazilian (Levy et al., 2013), US 1 to 3 year olds (Rojas-Sanchez et al., 1999), 1 to 3 year old Brazilians (Paiva et al., 2003) and 1 to 2 year old Colombians (Franco et al., 2005b) children respectively living in fluoridated communities. These

reasons might also be ascribed to why it was lower than 0.18 mg/kg bw/day for 1 to 3 year old Mexican children living in fluoridated areas (Martínez-Mier et al., 2003). The TDFI of 1 to 3 year old Brazilian children was 0.13 mg/kg bw/day (De Almeida et al., 2007) similar to the 0.14 mg/kg bw/day found in 4 year olds in this present study.

The percentage contribution of diet to TDFI can vary greatly, depending on the population and their dietary and oral health related habits, from 28% for 1 to 3 year old Mexicans (Martínez-Mier et al., 2003) to 99% for 3 to 4 year old Palestinians (Abuhaloob et al., 2015) all living in fluoridated areas. In this current study, the dietary contribution to TDFI was 71% and 75% for 4 and 8 year olds respectively probably due to differences in dietary and tooth cleaning behaviours. The 71% contribution among 4 year olds was higher than 45%, 28 – 36% and 34% reported for 1 to 2 year old Brazilians (Lima and Cury, 2003), 1 to 3 year old Mexicans (Martínez-Mier et al., 2003) and 4 to 5 year old Colombians (Franco et al., 2005b) living in fluoridated areas. It was also higher than 30.6%, 30% and 51.5% reported for 3 to 4 year olds in New Zealand (Guha-Chowdhury et al., 1996), 2 to 3 year old Colombians (Franco et al., 2005a) and 2 to 6 year Brazilians (Levy et al., 2013) living in non-fluoridated areas respectively. Conversely, it was lower than 75.4% and 99.02% reported for 3 to 5 year old Chilean and 3 to 4 year old Palestinian children living in fluoridated areas respectively and the 80-85.7%, 90.7% and 99.96% reported for 4 year old Iranians (Zohouri and Rugg-Gunn, 2000b), 3 to 5 year old Japanese (Murakami et al., 2002) and 3 to 4 year old Palestinians (Abuhaloob et al., 2015) living in non-fluoridated areas respectively. Compared with previous studies (Miziara et al., 2009, Levy et al.) where FFQ was used to collect dietary intake among 2 to 6 year olds in fluoridated and non-fluoridated areas, the percentage contribution of diet to TDFI in this current study was higher. There was no known study among 8 year olds to compare the percentage contribution of diet to TDFI but the 75% dietary contribution to TDFI in this current study was higher than 53 – 65% and 33.3% reported for UK 6 to 7 year olds (Maguire et al., 2007) and 6 to 7 year old Brazilians (Pessan et al., 2003) living in fluoridated areas. The differences in the number of participants, age group studied, where the study was undertaken, and dietary and tooth cleaning habits might be the reason for differences in the reported percentage contribution of diet to TDFI.

The contribution of F toothpaste to F intake has been highlighted in many studies. In the present study a contribution of 29% and 25% from toothpaste to TDFI was estimated for 4 and 8 years respectively due to higher toothpaste ingestion among the younger children. A contribution ranging from 39% to 87% was reported for US 1 to 3 year olds (Rojas-

Sanchez et al., 1999) and 1 to 3 year old Brazilians (De Almeida et al., 2007) in fluoridated areas; values higher than the amount found in this current study and possibly due to differences in tooth cleaning habits. The contribution to F intake from toothpaste among 4 year olds in this current study was only higher than 24.6% reported for 3 to 5 year old Chilean children (Villa et al., 2000) living in fluoridated areas. It was lower than the 69.4% and 70% reported for 3 to 4 year New Zealand (Guha-Chowdhury et al., 1996) and 2 to 3 year old Colombian (Franco et al., 2005a) children living in non-fluoridated areas. However, it was higher than 0.04%, 9.3% and 14.3-20% reported for 3 to 4 year old Palestinian (Abuhaloob et al., 2015), 4 year old Iranian (Zohouri and Rugg-Gunn, 2000b) and 3 to 5 year old Japanese (Murakami et al., 2002) children living in non-fluoridated areas respectively. The contributions of 35% to 67.7% reported for UK 6 to 7 year olds (Maguire et al., 2007) and 6 to 7 year old Brazilians (Pessan et al., 2003) living in fluoridated areas were higher than that proportion estimated for 8 year olds in this present study (25%). Similarly, a contribution of 57% reported for 6 to 7 year old UK children living in non-fluoridated areas was also higher than in this present study. Variations in the tooth cleaning habits of study participants in terms of the frequency and amount of toothpaste used and F analytical methods employed might be the reason for the observed differences in the percentage contribution of toothpaste to TDFI. In addition, poor access to toothpaste as well as high intake of F from diet as in Area 2 where toothpaste was only 6% of TDFI might be another reason for the differences

Overall, the percentage contribution to TDFI from drink, food and toothpaste respectively was 17%, 54% and 29% in 4 year-olds and 22%, 54% and 25% in 8 year-olds. The contribution to TDFI from food was quite high, while from drink it was lower. This is because larger amounts of food compared to drink were consumed, and the F concentrations of foods were higher than in drinks. In summary, the percentage contribution to TDFI from diet (food and drinks) was 71% and 76% for 4 and 8 year-olds respectively indicating that diet was the major contributor to TDFI as reported in other previous studies, 65% 75.4% and 99.02% for UK 6 – 7 year olds (Maguire et al., 2007), 3 – 5 year-old Chilean (Villa et al., 2000) and 3-4 year-old Palestinian (Abuhaloob et al., 2015) children respectively. In contrast, some previous studies reported that toothpaste was the major contributor to TDFI; 55%, 66% and 64% – 72% reported for 1.8 – 2.6 year-old Brazilian (Lima and Cury, 2001), 4 – 5 year-old Colombian (Franco et al., 2005b) and 1.3 – 3 year-old Mexican (Martínez-Mier et al., 2003) children respectively, however these

studies only estimated toothpaste contribution rather than analysing tooth brushing expectorate to estimate toothpaste ingestion.

6.5.13 Urinary fluoride excretion

The corrected urine volume for 4 year olds 394 ml/day was lower than 440 ml/day, 498 ml/day and 499 ml/day reported for 3 year old Iceland, UK and Irish children respectively (Ketley et al., 2004). Similarly, it was lower than 540 ml/day, 646 ml/day and 726 ml/day reported for 3 year old Dutch, Finnish, and Portuguese children respectively (Ketley et al., 2004). It was also lower than 449 ml/day and 568 ml/day reported for 4 year old UK children who lived in fluoridated area of the north-east of England (Rugg-Gunn et al., 1993) and 3-6 year old German children living in a salt fluoridation area (Haftenberger et al., 2001). The corrected urine volume for 8 year olds (618 ml/day) was within the range reported for children aged 3-7 years in the literature (Zohouri and Rugg-Gunn, 2000b, Ketley and Lennon, 2000, Villa et al., 2000, Zohouri et al., 2006a). It was higher than 482 ml/day, 495 ml/day and 534 ml/day reported for 6-7 year old UK children living in sub-optimal, optimal and non-fluoridated areas respectively (Maguire et al., 2007). The observed differences in urine volumes seen in F excretion studies undertaken among these children due to volumes of fluids consumed as a result of dietary habits, age, and local climate.

The mean urinary F excretion (UFE) of 0.50 mg/day for 4 year-olds was higher than 0.15mg/day reported as the standard UFE for low exposure (0.02 mgF/kg) and 0.33-0.45 mg/day reported for this age group who are optimally exposed (0.05-0.07 mgF/kg) to F (WHO, 2014). It was within the range of 0.15 mg/day to 0.75mg/day reported for four, 0.2-1.2 year old American who were formula-fed (Ekstrand et al., 1994b) and 4-6 year old American who lived water fluoridated area (Baez et al., 2000). It was fairly similar respectively to 0.48 mg/day and 0.53 mg/day reported for 3-6 year old German children living in salt fluoridated area (Haftenberger et al., 2001) and 3-5 years Chilean children who consumed fluoridated water and F supplement (Villa et al., 1999). It was higher than 0.16-0.21 mg/day reported in a multi-country study for 1.8-5.2 year old European children (Ketley et al., 2002, Ketley et al., 2004). It was higher than 0.32 mg/day, 0.34mg/day and 0.35 mg/day reported for 6-7 year old UK (Maguire et al., 2007), 4 year old Iranian (Zohouri and Rugg-Gunn, 2000b) and 3-5 years Chilean (Villa et al., 2000) children living in sub-optimal and optimally fluoridated areas. It was respectively lower than 0.55 mg/day and 0.59-0.62 mg/day reported for 4 year old Sri-Lankans who lived in optimally

fluoridated water area (Rugg-Gunn et al., 1993) and 2-7 year old Brazilian children who lived in >1.5 ppm water area (Forte et al., 2008). Differences in physical inactivity, F metabolism, and the type of diet consumed could be the reason for the observed differences in mean urinary F excretion. Depending on the balance of several factors, exercise could be associated with either decreased or increased circulating F levels (Whitford, 1996b) and physical inactivity may alter the pattern of F excretion (Buzalaf and Levy, 2011). Ingestion of diets containing NH_4Cl and $NaHCO_3$ can result in acidification and alkalinisation of urine respectively (Buzalaf and Levy, 2011). Acidification of urine will lead to decreased urinary F excretion and a subsequent increase in F retention while alkalinisation of urine will result in increased urinary F excretion and therefore decreased F retention. Long term diet-induced changes in urinary pH could therefore decrease (alkaline urine) or increase (acidic urine) the risk of dental fluorosis (Whitford, 1997).

The mean F excretion of 0.55 mg/day for 8 year olds was higher than the 0.21 mg/day, the WHO standard for low exposure while it is within 0.47-0.65 mg/day, the WHO standard for optimal range of F exposure for this age group (WHO, 2014). It was lower than the 0.93-3.20 mg/day reported for 8-9 year olds living in optimal and high water F areas of Mexico (Grijalva-Haro et al., 2001) and higher than the 0.22 mg/day reported for 1-9 year old Kuwaiti children living in non-fluoridated area (Akpata et al., 2014). Differences in the total daily F intake among the children in these studies is most likely to be the main reason for the difference in mean urinary F excretion seen, when compared with the present study.

For both 4 and 8 year-olds, on the basis of body weight, the mean UFE was 0.03 mg/kg bw/day, higher than 0.01 mg/kg bw/day reported as standard for those exposed to low F but within 0.02-0.03 mg/kg bw/day reported for those optimally exposed to F. For both age groups, only Area 2, showed a higher mean UFE (0.05 mg/kg bw/day) for 4 and (0.6 mg/kg bw/day) for 8 year-olds compared to optimal range (0.02-0.03 mg/kg bw/day) while it was within this optimal range in other areas. The reason for this higher mean UFE in Area 2 was due to the higher mean TDFI.

Based on individual urinary F excretion measurements, the range of urinary F excretion found in 4 year-olds (0.074 to 1.84 mg/day) and 8 year-olds (0.017 to 5.20 mg/day) suggests differences in F intake which depend on what they are eating or drinking and their tooth brushing habits. It also shows that at an individual level some of the children did not receive optimal F exposure while some children were exposed to excessive F. Some of the variation seen in the urinary F excretion in both age groups might be due to some

inaccuracies in estimating F excretion but this is doubtful because urinary F excretion was validated i.e. reporting the completeness of 24h urine samples collected and only including those meeting appropriate inclusion criteria (WHO, 2014) in the analysis. Furthermore when quality control of the urine analytical method was undertaken, measurement of F in urine was reproducible because the mean difference between first and second analysis was 0.007 (-0.247 to 0.233 µgF/ml) at p=0.95.

6.5.14 Fluoride retention

To report F retention for study participants in this present study, the faecal excretion of F was estimated based on the study by Ekstrand and colleagues (Ekstrand et al., 1984) who found that almost 10% of daily ingested F was excreted through faeces in infants aged 8 to 28 weeks living in optimally fluoridated area. On this basis, the daily fractional F retention (DFFR) was estimated as 46% and 54% for 4 and 8 year olds in the present study. For 4 year olds, it was lower than 54% reported for 3 to 5 year old Chilean children living in optimally fluoridated areas (Villa et al., 2000) while it was higher than 12.5%, 11% and 15% reported for formula-fed US infants (Ekstrand et al., 1994a), 4 year old Iranian (Zohouri and Rugg-Gunn, 2000b) and North American (Brunetti and Newbrun, 1983) children respectively primarily due to the level of F exposure and the age of the children; i.e. whether they were likely to be undergoing a period of active growth. The DFFR of 8 year olds (54%) was slightly lower than the 58% reported for 6 to 7 year old UK children living in optimally fluoridated areas while it was slightly higher than the 50% reported for 6-7 year-old UK children living in sub-optimally fluoridated areas (Maguire et al., 2007) probably because of differences in geographical locations and dietary practices, but also potentially due to differences in the stage of growth of these 8 year old Nigerian compared with 6-7 year old UK children. The DFFR of two 8 year olds was in negative balance while the fraction of ingested F excreted in their urine was greater than 100%. Negative balances have been reported in breast-fed infants with F intake of 5 to 19 ug/day (Ekstrand et al., 1984). Negative balances might be due to differences in the rate of F uptake from bones and teeth which depends on the stage of skeletal development of children. Uptake of F is faster in newly formed bones than in the mature bones, therefore F retention is greater during period of rapid growth (Whitford, 1994b). Reduction in F intake with subsequent reduction in plasma F concentration could result in a negative F balance. When F intake is reduced, F ion is mobilised from calcified tissues and become available for urinary excretion (Whitford, 1994b). Another reason for the negative F balances in children is the difference

in the dietary composition. Calcium and magnesium can form insoluble complexes with F in diets thereby reducing F absorption and uptake into teeth and bone. A diet rich in protein and fat increases the absorption and results in an increase in the proportion of F intake retained in the body (Cerklewski, 1997).

6.5.15 Relationship between F exposure and F excretion or retention

In this study, there was a relationship between F exposure (total daily F intake) and urinary F excretion, as the total daily F intake increased, the amount of F excreted and the F retention (% of F intake retained by body) also increased. However, this relationship was not strong or close because of confounding variables that could limit F intake and excretion such as genetics, malnutrition and other environmental factors. It might also be due to the method of assessing TDFI in this present study where food frequency questionnaire was used to provide estimate rather than actual intake. In addition, the wide variation in both TDFI and UFE might not make the correlation between F intake and excretion to be close. The proportion of total daily F intake retained by the majority of children in this study increased sharply at a total daily F intake lower than approximately 2.5 mgF/day and 3.0 mgF/day for 4 and 8 year-olds respectively (Figures 6.3 and 6.5). For TDFI values higher than approximately 2.5 mgF/day and 3.0 mgF/day for 4 and 8 year-olds respectively the estimated FFR tended to reach limiting constant values independent of how high the TDFI was. These findings are at variance with the result of a study (Villa et al., 2010) that investigated the relationships between total daily F intake, urinary F excretion and F retention among 0.15 to 7 year old Chilean children where the proportion of total daily F intake retained by the majority of children increased sharply at a total daily F intake lower than approximately 0.5 mg/day.

6.5.16 Relationship between nutritional deficiency and F excretion or retention

Some studies have suggested that malnutrition can increase the occurrence of dental fluorosis (Rugg-Gunn et al., 1997, Yoder et al., 1998) because of a lack of F complexes in the dietary intake, due to lack of dietary intake as a whole, resulting in increased F absorption from the GI Tract. In the present study the relationships between nutritional deficiencies reported as stunting and wasting and F exposure parameters were not statistically significant. The F intake, UFE and DFR was not lower among 4 year olds who were stunted or wasted compared with healthy children. This could mean that stunted or wasted 4 year olds may not be at higher risk of dental fluorosis than well-nourished children. The F intake and DFR was higher, while the UFE was lower among 8 year olds

who were stunted compared with well-nourished 8 year olds which may expose them to a higher risk of non-dental problems associated with F ingestion. However, in this 8 year old age group, the amount of F intake or excreted or retained among those who were stunted is less likely to lead to dental fluorosis since they have past the main period of tooth development. The F intake and F retention was lower among 8 year olds who were malnourished while the amount of F excreted was higher among those who were malnourished. This could mean that malnourished 8 year olds may not be at higher risk of dental fluorosis than well-nourished children. Further studies would be useful to investigate the effect of nutritional status on F intake, excretion and retention.

6.5.17 Conclusions

- The relative contribution of drink, food and toothpaste to TDFI was respectively 17%, 54% and 29% for 4 year olds while the corresponding contributions for 8 year olds were 21%, 54% and 25%.
- For 4 and 8 year-olds, only Area 2, showed a higher mean TDFI (0.39, 0.33) mg/kg bw/day respectively compared to optimal range of 0.05-0.07 mg/kg bw/day while it ranged between 0.04-0.08 mg/kg bw/day in other areas.
- For both age groups, only Area 2, showed a higher mean UFE (0.05 mg/kg bw/day) for 4 and (0.6 mg/kg bw/day) for 8 year-olds compared to optimal range (0.02-0.03 mg/kg bw/day) while it was within this optimal range in other areas.
- The wide range of urinary F excretion found between 4 year olds (0.074 to 1.84 mg/day) and 8 year olds (0.017 to 5.20 mg/day) suggested that at an individual level most of the children did not receive optimal F concentration, while some children received high exposure to F.
- The relationship between TDFI and urinary F excretion was linear, positive and statistically significant for both 4 ($\rho=0.41$; $p=0.001$) and 8 ($\rho=0.57$; $p<0.001$) year-olds.
- The relationship between TDFI and fractional F retention was linear, positive and statistically significant for both 4 ($\rho=0.56$; $p<0.001$) and 8 ($\rho=0.50$; $p<0.001$) year-olds. At a threshold value for TDFI of approximately 2.5 mgF/day and 3.0 mgF/day respectively for 4- and 8- year olds, the estimated FFR tends to reach a limiting constant value independent of how high the TDFI was.
- There was no statistically significant relationship between nutritional deficiency and F excretion and retention for both 4 and 8 year-olds ($p>0.05$).

Chapter 7 Phase 3 of the study – Single Nucleotide Polymorphism

7.1 Introduction

Numerous evidence indicates that fluoride is effective in the prevention and control of dental caries predominantly through its topical rather than systemic effect (Featherstone, 1999, Zohoori et al., 2014). Topical oral exposure to low levels of fluoride can help prevent demineralisation and promote remineralisation of early carious lesions (Zohoori et al., 2014). However, excessive systemic fluoride consumption or ingestion during tooth development can result in dental fluorosis. In a review of fluoride and dental caries prevention in children, Lewis (2014) reported that on the basis of strong evidence, community water fluoridation has markedly decreased rates of dental caries globally since it was first implemented in the mid-20th century, however, the degree of dental fluorosis in the population has been shown to be directly related to the fluoride concentration in drinking water. A way of reducing some of the burden of dental fluorosis is to identify susceptible populations within the community before water fluoridation to prevent dental caries. A polymorphism in the gene that codes for the Collagen 1 (A2) (COL1A2) protein has been identified as being associated with an increased risk of developing dental fluorosis in populations exposed to high fluoride (Huang et al., 2008). This potential biomarker could be used to identify high-risk populations that are genetically susceptible to dental fluorosis which would help to guide clinical and public health decisions concerning the optimal use of fluoride at the community as well as at the individual level. This chapter described the materials and methods used to carry out phase 3 of the study and it also described the results of this phase of the study.

7.2 Aim

To determine the prevalence of a single nucleotide polymorphism (SNP) within the COL1A2 gene among a subgroup of the Phase 1 study participants with and without dental fluorosis.

7.3 Materials and methods

7.3.1 Introduction

After collecting information on fluoride exposure from diets and toothpaste and urinary fluoride excretion from the study participants who participated in Phase 2 of the study, buccal mucosa swabs were taken from them for gene sequencing to determine the

relationship between the COL1A2 SNP and the occurrence of dental fluorosis in Phase 3 of the study.

7.3.2 Phase 3 Sample

The 144 four and eight year old study participants who were a subsample of the 624 study participants (302 4-year-olds) and (322 8-year-olds) who took part in Phase 1 and 2 of the study were also randomly recruited into the Phase 3 of the study but data were collected from them on first come first recruit basis. Their recruitment was based on their parents or legal guardians' consent allowing them participate in Phase 2 and Phase 3 of the study. In addition, they were recruited into Phase 2 and 3 of the study until the sample was complete.

7.3.3 Preparation for Phase 3 of the study in the UK

7.3.3.1 Trainings undertaken by researcher

Prior to DNA extraction in the Oral biology Laboratory of the Newcastle University, necessary training on biological and chemical safety of basic laboratory techniques provided by the Health and Safety unit of the Newcastle University was attended by the researcher. One week training on DNA extraction from 5 samples was undertaken.

7.3.3.2 Risk and BioCOSHH assessments

These two assessments were carried out to ensure that health and safety issues were considered before any swabs were stored in the laboratory as well as before the commencement of the laboratory analysis.

7.3.3.3 Procurement and transport of the research materials from the UK to Nigeria

The non-invasive swab matrix was procured from Isohelix, Cell Projects Ltd, UK. The RNALater Ambion solution was procured from life technologies while the biological bag (Category B UN3373) for transporting the buccal mucosa swab was procured from Air Sea Containers Ltd, UK. The 10 ml bulb end plastic pipette was procured from Fisher Scientific Ltd.

7.3.4 Preparation for Phase 3 in Nigeria

7.3.4.1 Training of the research recorder in Nigeria

The research recorder who was recruited for the study was trained on how to provide assistance during the buccal swab procedure, to ensure that study participants for Phase 3 were easily identified and that consent had been given by parents or legal guardians. The recorder was shown the materials that were to be used to collect the buccal mucosa swab,

namely: the non-invasive swab matrix, tube and cap, RNALater solution, bulb end pipette and ice packs in a cold bag. The recorder was trained on how to open the swab matrix, give and take the swab matrix from the operator, label the tube, insert the swab head into the tube, dispense RNALater into the tube, cap the tube and place the tube in a cold bag containing ice packs. The recorder was later asked to re-iterate and demonstrate it and necessary corrections were made until she was able to complete the task correctly.

7.3.4.2 Transport of materials and research team

The research van was used to transport the research team and materials needed to the selected nursery and primary schools in line with the work schedule. The items for the buccal mucosa swab taking were placed on the clinical dental examination table.

7.3.5 Genetic Data collection, preparation and analysis

7.3.5.1 Swab sample collection

After carrying out the clinical dental examination, the research recorder checked the field work book to ascertain whether the study participant had been recruited for Phase 3 of the study. After confirming that the participant was recruited for Phase 3 of the study and that parents or legal guardians had consented to allow their child or ward to participate in Phase 3, the participant rinsed the oral cavity with clean water to ensure that high quality buccal mucosa cells were taken. After rinsing the oral cavity, a buccal mucosa swab was taken by rubbing a non-invasive swab matrix (Isohelix DNA Buccal Swab – SK-1S) on the mucosa of the cheek 5 to 10 times. After taking the swab, the shaft of the swab matrix was snapped just above the swab head and placed into a labelled 5ml tube containing 3ml RNAlater solution a storage media that stabilizes and protects cellular RNA. The tube was then sealed with a cap and placed in the cold bag containing an ice pack before transporting to a -20^{o}C freezer at the Oral Pathology Laboratory, Dental School, University of Ibadan, Nigeria where the samples were stored frozen.

7.3.5.2 Transport of the oral mucosa swab samples from Nigeria to the UK

The frozen buccal mucosa swab samples were transported to the Fluoride Research Laboratory, Newcastle University United Kingdom using dry ice in a Category B UN3373 Air Sea Biobag-1 95 Kpa 235 x 155 mm (120 mm opening). On arrival, the swab samples were immediately stored in 4^{O}C fridge in the Fluoride Research Laboratory before they were transported to 4^{O}C fridge in the Oral Biology Laboratory, Newcastle University 2 days before the commencement of the molecular biology techniques.

7.3.5.3 Laboratory analysis of the buccal mucosa swab samples

The two molecular biology techniques carried out were DNA extraction and DNA amplification by polymerase chain reaction (PCR). RNA was not prepared from samples as the integrity was not of high enough quality for downstream analysis. All consumables and solutions were sterilised by double high pressure and temperature autoclave. All reagents were obtained from Life technologies (Life Technologies, UK) unless stated otherwise, and were of molecular biology grade (i.e. DNase free where applicable).

DNA Extraction: DNA extraction is the removal of deoxyribonucleic acid (DNA) from cells such as buccal mucosa cells in a purified form for further investigations. It is often an early step in many diagnostic processes used to detect diagnose diseases and genetic disorders. After the DNA is extracted it can be amplified by PCR and sequenced (to investigate the order of nucleotides – adenine, guanine, cytosine and thymine within the DNA template produced) for comparison with existing sequences available in the public database (National Centre for Biotechnology Information (ncbi) – www.ncbi.nlm.nih.gov). The process of DNA extraction involves:

Preparation of lysate: The lysate was prepared using the following materials: PureLink Proteinase K, PureLink Genomic Lysis/Binding Buffer, PureLink Genomic Digestion Buffer, Phosphate Buffered Saline (PBS), Sterile DNase-free microcentrifuge tubes, water bath and buccal mucosa sample. Each swab sample was taken from the 4^{o}C fridge and used to prepare a lysate. The lysate was prepared by centrifugation at 4^{o}C at 1000Xg of the swab sample into a pellet in the well labelled tube containing RNA later solution for 5 minutes. The RNALater was carefully poured out of the tube leaving out the pellet and little amount of RNALater in the tube. Then 200µL of PBS was added to the cells in the pellet and mixed thoroughly by vortexing for 5 seconds. Into a well labelled sterile microcentrifuge tube, 20 µL of proteinase K was dispensed and 200 µL of lystate (cells and PBS) transferred into this microcentrifuge tube and mixed thoroughly by vortexing for 5 seconds. An equal volume of genomic lysis/binding buffer was added to the lysate and proteinase K and mixed thoroughly by vortexing for 5 seconds. The vortexed genomic lysis/binding buffer, lysate and proteinase K was incubated at 55^{o}C in a water bath for 10 minutes and then 200 µL of 96-100% ethanol was added before mixing well by vortexing for 5 seconds.

Binding of the DNA: The binding of the DNA was carried out by the addition of 600µL of the lysate prepared with PureLink Genomic lysis/Binding buffer and ethanol into a well-labelled PurLink spin column and then centrifuged at 12,000Xg for 1 minute at room

temperature in multiple loading of 200 µL of the lysate. Each time centrifuging was carried out, the collection tube was discarded and the spin column was placed into a clean sterile PureLink collection tube.

Washing of the DNA: After centrifuging the spin column, the DNA was washed by adding 500 µL of Wash Buffer 1 to the PureLink spin column and the column was centrifuged at 8,000Xg for 1 minute at room temperature. The collection tube was discarded and the spin column was placed into a clean PureLink collection tube after the centrifugation. Furthermore, 500 µL of Wash Buffer 2 was added to the PureLink spin column and the column was centrifuged at maximum speed of 13,000Xg for 3 minutes at room temperature. After this centrifugation, the collection tube was discarded and the spin column placed in a sterile 1.5 mL centrifuge tube.

Addition of buffer: After washing the DNA, 25 µL of PureLink Genomic Elution Buffer was added to the column and incubated at room temperature for 1 minute. After the incubation, the column was centrifuged at maximum speed of 13,000Xg for 1 minute at room temperature. To recover more DNA, a second elution was performed. The spin column was removed and discarded while the purified DNA in the microcentrifuge tube was stored in a -4^{o}C fridge.

Purification of the DNA: Finally, the amount of DNA purified was quantified using a spectrophotometer attached to a computer containing ND-1000, software for measuring DNA. The pedestal of the spectrophotometer was cleaned and 1 µl of deionized Distilled water was loaded onto it to initialize and calibrate the meter. After the initialization and calibration, 1 µl of DNA sample was pipetted onto the pedestal of the meter and the amount of DNA was measured. The Concentration of DNA (µg/L) and the absorbance ratio (260/280) were recorded.

PCR Amplification of the purified DNA: Amplification of DNA is the process by which multiple identical copies (replicates) of a DNA sequence is produced. One method by which DNA can be amplified is by polymerase chain reaction (PCR). PCR amplification is capable of producing enormous identical copies of a short DNA sequence from a single molecule of starter DNA. It amplifies a specific DNA (target) sequence lying between known positions (flanks) on a double-stranded DNA molecule. The process of PCR amplification involves:

Design of PCR primers: Oligonucleotide primers were generated based upon those previously used (Huang et al. 2008). Forward primer 5’

GGGATCCTCGGCCCCGCTGGAAAAGAA 3' and reverse primer 5' CCGAATTCACCTTTATCACCGTTTTTGCCA 3'. These primers generated a 500 base pair product with the SNP directly in the middle of the sequence. They were synthesised by the Integrated DNA services (IDT, UK).

Preparation of the stock primer: Stock concentrations of 100 µM of each oligonucleotide primer were made and a working stock concentration was achieved by diluting this 1:10 v/v with water, to give a final concentration of 10 µM.

Preparation of the reagents for PCR amplification: To 1 µg of genomic DNA (prepared as described in Section 7.3.5.3) 12.5 µl of Taq Master Mix (containing 2 X buffer, Taq, polymerase, Mg^{2+} and dNTPs; NE Bioline) was added. To the mixture 0.5 µM of Forward and 0.5 µM Reverse primer was added and water, up to a volume of 25 µl was added.

PCR Amplification: After optimisation at different annealing temperatures, it was decided that the samples would be incubated on as thermal cycler programmed to run at 95^{o}C for 5 minutes to activate enzyme, the separation was set at 95^{o}C for 30 seconds, the annealing was set at 58^{o}C for 30 seconds and the extension was set at 72^{o}C for 60 seconds (Table 7.2). PCR cycling was carried out for 30 seconds. PCR negative reactions were carried out at the same time, with no genomic DNA present.

Visualisation of DNA by Gel Electrophoresis: A casting tray was prepared by putting masking tapes at both ends of the tray. To produce 1.0% w/v agarose gel, 1 gram of agarose powder plus 100 ml of 1x Tris-Acetate-Ethylenediaminetetracetic acid (TAE) were dispensed into a beaker and thoroughly mixed. The mixture was nested in a microwave for 7 minutes thereby ensuring a proper mix with no bubbles. After mixing the agarose and TAE thoroughly, the solution was cooled to 60^{o}C. Gel red nucleic acid stain (Phenix RGB – 4103) which intercalates with DNA and allows visualisation under UV light was centrifuged for 5 seconds and 10 µL was added to the agarose gel at a concentration of 2µg/mL and the mixture was mixed properly until the agarose gel was homogenously red. The agarose gel poured into the casting tray. A well comb was inserted and the gel in the casting tray was then allowed to set on the bench for 15 minutes. Once set the casting tray was immersed into a gel box containing 1 X TAE buffer. In the trough of TAE buffer, the well comb was then removed and 5 µL of DNA hyperladder IV and V were each loaded into first well, 2 µL of 5X DNA gel loading buffer (50 mM Tris (pH 8.0), 5 mM EDTA, 50% glycerol (v/v), 50% bromophenol blue (w/v) mixed with 8 µL of DNA samples and loaded onto the agarose gel. Gels were run at 80 volts for 30 minutes. DNA was visualised

on a UV trans-illuminator and images were taken (Figure 7.1). Successful PCR reactions were visualised for 70 of the samples analysed and these DNA samples were sent to MWG-Eurofins, Milton Keynes, UK for gene sequencing using a sequencing primer (Table 7.1).

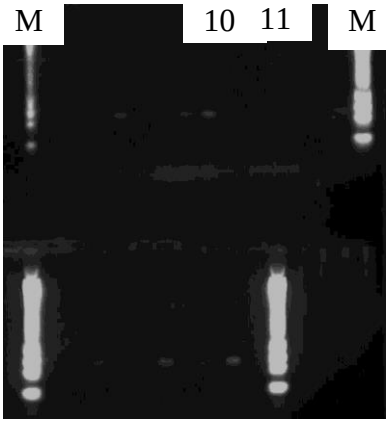

Figure 7-1: A representative gel image of PCR products run on a 1% agarose gel. Bands show a positive reaction. Lane M show marker (Hyperladder IV). A negative PCR reaction is shown in lane 10 on the second gel while a positive PCR is shown in lane 11

DNA sequencing: A sequencing primer was generated when the DNA was sequenced by MWG-Eurofins, Milton Keynes, U.K (Table 7.1). Sequencing was analysed by eye to establish whether the SNP was present or absent on the chromatogram. Heterozygous samples could be visualised as 2 peaks.

Primer sequences	Denaturation	Annealing	Extension	cycles
PCR forward primer - 5' gga aat atc ggc ccc gct gga aaa 3'	95ºC	58ºC	72	30 cycles
PCR reverse primer - 5' gtc cag cca atc caa tgt tgc c 3'	95ºC	58ºC	72	30 cycles
Sequencing primers – 5 GTCCAGCCAATCCAATGTTGCC 3’				

Table 7-1: The primer sequences and conditions for PCR of the various SNPs of human COL1A2 among study participants.

7.3.6 F concentration of drinking and cooking water samples

Data of F concentration of drinking and cooking water samples of Phase 3 study participants was obtained from Phase 1 (See Chapter 5).

7.3.7 Data analysis

Data were entered into Statistical Package of Social Sciences (SPSS) software for analysis. Frequencies and proportions were generated. Chi-square test was used to test association between categorical variables at ($p<0.05$). Binary regression analysis was undertaken to estimate the relationship between the dichotomous dependent variables (presence/absence

of dental fluorosis) and the explanatory independent variables (type of SNP and F concentration in drinking and cooking water) at $p<0.05$.

7.4 Results

7.4.1 Sample analysis

The DNA of 140 samples were extracted but only 70 (34 4-year-olds; 36 8-year-olds) were visible as a positive reaction by gel electrophoresis and therefore had quality DNA which were later sent for gene sequencing.

7.4.2 Pattern of distribution of single nucleotide polymorphism (SNP)

The position of possible SNP was identified on each of the sequencing chromatograms – at around base pair 270 of the sequencing chromatogram. The base associated at this position was recorded as either 'AA', 'CC' or AC.

Figure 7.2A represents a chromatogram from a child who was carrying the 'AA' base, Figure 7.2B represents a chromatogram from a child who was carrying the 'CC' base and Figure 7.2C represents a chromatogram from a child who was carrying the 'AC' base.

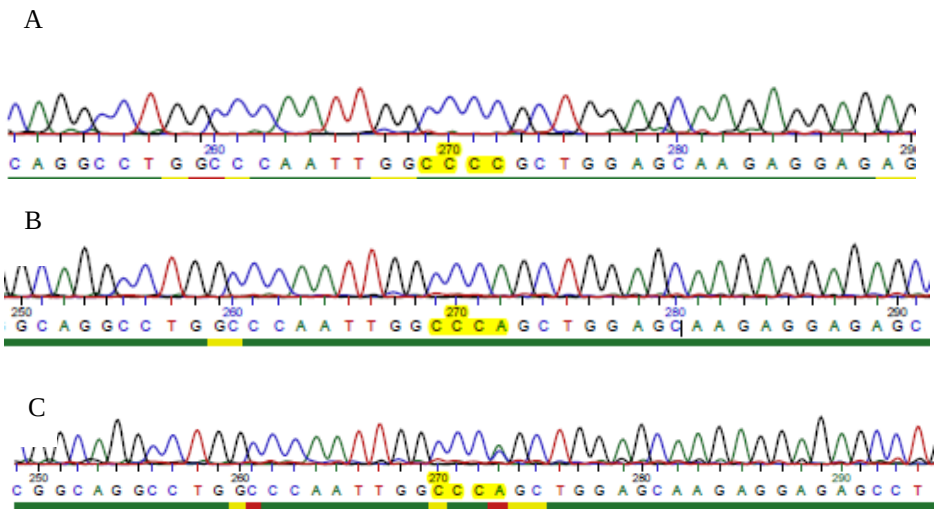

Figure 7-2: **DNA sequences showing the substitution region (SNP AA (A) or CC (B) or AC(C). The fourth letter highlighted in yellow represents the SNP.**

Table 7.2 shows the distribution of single nucleotide polymorphism. The majority of the study participants 37 (52.9%) had the heterozygote SNP AC.

SNP	Number	(%)
AC	37	(52.9)
AA	24	(34.2)
CC	9	(12.9)
Total	**70**	**(100.0)**

Table 7-2: Distribution of single nucleotide polymorphism among study participants (n=70).

The proportion of study participants whose drinking water or water used for cooking contained between 0.6-4.0 ppm was highest (33.3%) among those who had SNP CC and lowest (5.4%) among those who had SNP AC (Table 7.3).

SNP	Drinking water		Cooking water		Number (%)
	0 – 0.3 ppm No. (%)	0.6 – 4.0 ppm No. (%)	0 – 0.3 ppm No. (%)	0.6 – 4.0 ppm No. (%)	
AC	35 (94.6)	2 (5.4)	33 (89.2)	4 (10.8)	37 (100.0)
AA	21 (87.5)	3 (12.5)	21 (87.5)	3 (12.5)	24 (100.0)
CC	6 (66.7)	3 (33.3)	7 (77.8)	2 (22.2)	9 (100.0)
Total					**70 (100.0)**

Table 7-3: Distribution of single nucleotide polymorphism by F concentration in drinking and cooking water samples consumed by study participants (n=70).

7.4.3 Association between single nucleotide polymorphism and dental fluorosis among study participants

Although 33.3% of study participants who carry the hypothesis SNP, CC had dental fluorosis, there was no statistically significant association between the risk SNP (CC) and presence/absence of dental fluorosis (P=0.30, Likelihood ratio=2.41), however, 33.3% of study participants who had SNP CC had dental fluorosis.

SNP	Dental Fluorosis Present		Dental Fluorosis Absent		Total		P value
	No.	(%)	No.	(%)	No.	(%)	
AA	4	(16.7)	20	(83.3)	24	(100.0)	0.30
CC	3	(33.3)	6	(66.7)	9	(100.0)	
AC	12	(32.4)	25	(67.6)	37	(100.0)	
Total	**19**	**(100.0)**	**51**	**(100.0)**	**70**	**(100.0)**	

Table 7-4: Association between single nucleotide polymorphism and dental fluorosis among study participants (n=70).

Notes: Likelihood ratio = 2.41

7.4.4 Relationship between Single Nucleotide Polymorphism, F concentration in drinking and cooking water and dental fluorosis

The Hosmer and Lemeshow goodness-of-fit test indicated that the data fit the model with a Chi-Square value of 13.01 and p=0.11 for dental fluorosis in the primary and permanent teeth of study participants. This shows that the model predicted accurately since p>0.05 i.e. there was no mis-specification of the predictive capacity of the model. The Nagelkerle R^2 from the model was 0.17 i.e. 17% of the variability in the dependent variable is accounted for by the independent variables. The predicted odds of having dental fluorosis based on F concentration in drinking water and carrying the SNP AC was 2.50 (CI: 0.99-6.32) and 1.84 (CI: 0.92-3.64) respectively (Table 7.5) but F concentration in cooking water, presence of SNPs CC and AC were not statistically significant predictors (p>0.05)..

Predictors	**Dental fluorosis (R^2=0.17; % Predicted = 73.5%)**				
	B	**Sig (p)**	**Exp (B)**	**95% CI**	
				Lower	**Upper**
F Concentration Drinking Water (mg/l)	0.92	**0.05**	2.50	0.99	6.32
F Concentration Cooking Water (mg/l)	0.95	0.33	0.39	0.06	2.58
Single nucleotide polymorphism					
SNP CC	0.47	**0.09**	1.21	0.78	4.36
SNPAC	0.61	**0.08**	1.84	0.92	3.64

Table 7-5: Binary regression analysis model for dental fluorosis among 4 and 8 year olds (n=70).

7.5 Discussion

7.5.1 Design and sample

The study was designed to collect buccal mucosal swab to determine prevalence of a single nucleotide polymorphism (SNP) within the COL1A2 gene among a subgroup of the Phase 1 study participants with and without dental fluorosis. It was also designed to determine the influence of SNP, F concentration in drinking and cooking water on the occurrence of dental fluorosis. The sample was randomly recruited from the study participants from the 4 areas in Phase 1 of the study for whom there was information on the occurrence of dental fluorosis and F concentration in drinking and cooking water. One hundred and forty four parents or legal guardians of the study participants consented that their children or wards should participate in the study. This was higher than the 128 participants needed for the study because many parents or legal guardians appealed for inclusion of their children or wards after the required sample size was achieved. This was because they were excited

about their children or ward participating in a research that would potentially proffer some solutions to oral health problems in children.

7.5.2 Aim of the study

The aetiology of dental fluorosis is not well understood, although the main risk factors are generally accepted to be environmental e.g. high F intake during tooth development are implicated. There have been several indications of a potential influence of genetics on the susceptibility to dental fluorosis, however, less have been done to explore the effect of a gene polymorphism on susceptibility. Studies (Suuriniemi et al., 2003, Willing et al., 2003, Deng et al., 2003) have reported significant associations between bone phenotypes such as bone mineral density and content as well as specific genes including COL1A2. The findings from these studies similarly underscore the similarities between bone and dental tissues in terms of their biological makeup and fluorosis related-pathogenesis. In addition, there is evidence that F affects genetic pathways and products that regulate the development and mineralization of teeth thereby can alter the process of amelogenesis and dentinogenesis (Zhang et al., 2006, Yan et al., 2007). These factors resulted in plausible speculation that a genetic polymorphism of the COL1A2 gene which play a role in bone formation or pathogenesis may be considered when exploring the aetiology of dental fluorosis. A previous study (Huang et al., 2008) looking at an association between two COL1A2 polymorphisms and dental fluorosis in a Chinese population with high F exposure provided both support for the plausibility of a role for genetic factors in aetiology of dental fluorosis as well as preliminary evidence for a specific role for COL1A2. The authors reported an association between the COL1A2 PvuII polymorphism (rs 414408) and dental fluorosis only in high F areas which is suggestive of gene-environment interaction. However, since the China study was carried out in areas of 2ppm water F, further studies are needed to confirm this finding and to investigate the impact of this polymorphism in different study populations. Therefore, the aim of the study was to determine the prevalence of this SNP within the COL1A2 gene among a subgroup of the Phase 1 study participants with and without dental fluorosis. In addition, the study also aimed to determine the influence of SNP and F concentration in both drinking and cooking water in the occurrence of dental fluorosis. This study allowed us to look at a different population (Nigerian) at differing F doses.

7.5.3 Sample collection, preparation and analysis

Buccal mucosal swabs of study participants whose parents or legal guardians consented to allow samples taken for genetic analysis were undertaken after the participants were dentally examined on the examination chair. Prior to the dental examination and swab taking participant's mouth was rinsed after tooth brushing with F toothpaste. This ensured that debris that could contaminate the swab was removed before the swab taking. The swab was placed in tube containing RNA later solution after swab taking to stabilize and protect the cell. The tube was then sealed with a cap and placed in the cold bag containing an ice pack before transporting to a -20^{o}C freezer where the samples were stored frozen. Samples were later transported to Newcastle University using dry ice and samples stored in -20^{o}C before genetic analysis. Transport of the buccal swabs from Nigeria to the UK was challenging because of the 4 to 5 hour delays by customs at the airports. The delays resulted in samples starting to defrost before their arrival at Newcastle University but did not affect the samples since RNAlater, the storage media doesn't actually need to be frozen. Prior to sample preparation and analysis in the Biology laboratory at Newcastle University, samples were stored at 4^{o}C. The DNA of the 144 samples was extracted but 70 samples had quality DNA and were sent for gene sequencing.

7.5.4 Pattern of distribution of single nucleotide polymorphism

In this present study, the majority of the study participants (52%) had heterozygous AC genotype of COL1A2 *Pvu*II while 34% AA and 12% had CC of COL1A2. The proportion of study participants whose drinking water or water used for cooking contained between 0.6-4.0 ppm was highest among those who had SNP CC and lowest among those who had SNP AC. Distribution of genotype frequencies in *Pvu*II was similar in the control subjects to reports in previous studies (Huang et al., 2008, Ba et al., 2011) (Table 7.6), however interestingly, there was a lower proportion of risk alleles and heterozygous genotypes in the cases which may account for lack of statistical significance.

Studies	**Country**	**Age (y)**	**n**	***Pvu*II SNP**		**Cases No. (%)**	**Control No. (%)**	**P value**
Huang et al. (2008)	China	8-12	75 (Cases) 165 (Controls)	PP Pp Pp	CC AC AA	14 (18.7) 24 (32.0) 37 (49.3)	18 (10.9) 75 (45.5) 72 (43.6)	0.084
Ba et al. (2011)	China	8-12	74 (Cases) 163 (Controls)	PP Pp pp	CC AC AA	15 (20.3) 27 (36.5) 32 (43.2)	30 (18.4) 77 (47.2) 56 (34.4)	0.285
Present study	Nigeria	4 & 8	19 (Cases) 51 (Controls)	PP Pp	CC AC	3 (15.8) 12 (63.2)	6 (11.8) 25 (49.0)	0.362

				pp	AA	4 (21.0)	20 (39.2)	

Table 7-6: Comparison of genotype frequency of *Pvu*II SNP between Nigerian children and other population

7.5.5 Association between SNP, F concentration in water and dental fluorosis

There was no statistically significant association between single nucleotide polymorphism and presence/absence of dental fluorosis, however, 33.3% and 32.4% of study participants who respectively had SNP CC and AC had dental fluorosis. From previous study (Huang et al., 2008) homozygous CC genotype of COL1A2 *Pvu*II was the risk genotype only among children who lived in endemic fluorosis and high F concentration areas but in the present study this was not the case possibly because very small numbers of participants living in high F areas carried this allele. Fluoride concentration in drinking water, presence of SNPs CC and AC had a tendency towards statistical significance as predictors of dental fluorosis; the risk of having dental fluorosis was respectively 2.50 (p=0.05) and 1.84 (p=0.08) times with increasing F concentration in drinking water and presence of SNP AC. The very small number of children living in high F areas might be the reason for the slightly higher risk and more statistically significant level of having dental fluorosis from F concentration in drinking water than the presence of SNP AC. The risk of having dental fluorosis was higher in the presence of SNP AC than SNP CC and might have been due to a higher proportion of the children carrying the former genotype than the latter. Of the 19 children who had dental fluorosis, 15 carried a C allele (either as CC or AC), so it seems that C allele in SNP CC and AC appears to be the risk allele for dental fluorosis.

Huang and colleagues (Huang et al., 2008) studied the interactions between COL1A2 gene and dental fluorosis in high and low water F areas while this current study looked at the interactions between the gene and F concentration in drinking and cooking water which provided information on actual F exposure from water. Studying the interactions between SNPs and F concentration in water alone cannot provide adequate information about gene-environment interaction in the occurrence of dental fluorosis since there are other influential environmental factors in aetiology. Therefore there is a need for further studies on the relationship between SNPs and other environmental factors in the occurrence of dental fluorosis. In addition, there is also a need to undertake a further study with a bigger population. COL1A2 SNPs may be a useful marker for the differential risk of dental fluorosis, which being a complex condition is likely influenced by several genes, therefore, further investigation on other polymorphism of COLIA2 gene and other candidate genes influencing dental fluorosis may be useful. Although the mechanisms of the underlying

relationship between COL1A2 *Pvu*II polymorphisms and dental fluorosis risk are not yet understood, studies have reported that individuals with the homozygous CC genotype had higher risk of fracture compared to those with the homozygous AA genotype (Suuriniemi et al., 2003) and lower bone mineral density/bone mineral content (Lau et al., 2004). The relationship between SNP and the severity of dental fluorosis was not explored in this current study. A previous study (Huang et al., 2008) that explored these variables reported that children with homozygous CC had a higher mean Dean's score compared to children with homozygous AA ($p<0.05$), however, the risk did not increase with severity of dental fluorosis. The authors in the study speculated that the high risk was driven by severe dental fluorosis.

Other studies (Huang et al., 2008, Wen et al., 2012) have explored the interaction between different SNPs and bone or parathyroid hormone metabolism and pathogenesis. This has stimulated the interest of some other authors to study the interaction between the different SNPs and dental fluorosis. Some authors have reported that calcium or bone metabolism-related genes such as oestrogen, calcitonin, osteocalcin might be associated with dental fluorosis (Ba et al., 2011). There are controversies over the relationship between the different SNPs and dental fluorosis. Huang and colleagues (Huang et al., 2008) reported no relationship between COL1A2 *Rsa*I polymorphism and dental fluorosis. Similarly, Wen and colleagues (Wen et al., 2012) reported no correlation between dental fluorosis and PTH *Bst* BI polymorphism, however, serum osteocalcin might be a more sensitive biomarker for detecting early stages of dental fluorosis. Similarly, a study on the association between osteocalcin gene polymorphism and dental fluorosis among children exposed to fluoride in China showed that osteocalcin HindIII polymorphisms may not be a useful genetic marker for differential risk of dental fluorosis among children in China (Ba et al., 2009a). Association of dental fluorosis with polymorphisms of oestrogen receptors among 8-12 year olds Chinese children showed that ESR gene RsaI and XbaI polymorphisms may be associated with the risk of dental fluorosis in a high-F-exposed population (Ba et al., 2011). However, a previous study (Wang et al., 2010) showed no association correlation between ER RsaI genotype and dental fluorosis.

7.6 Conclusions

- The majority of the study participants had the heterozygote SNP AC genotype of COL1A2 *Pvu*II.

- Fluoride concentration in drinking water (p=0.05) and the SNPs CC (p=0.09) and AC (p=0.08) in COL1A2 had a tendency towards statistical significance as predictors of dental fluorosis in both primary and permanent teeth of 4 and 8 year olds.
- The C allele in COL1A2 SNPs CC or AC may be a useful genetic marker for the differential risk of dental fluorosis. Further investigations are needed to confirm this finding in larger and different study populations.

Chapter 8 Exploring the relationships between environmental and genetic factors in the occurrence of developmental enamel defects and overall discussion

8.1 Introduction

Previous chapters (5, 6 and 7) have presented both univariate and bivariate analysis of dental health, nutritional status, fluoride exposure and presence of single nucleotide polymorphism (SNP) among study participants. This chapter presents a multivariate analysis of the environmental and genetic predictors identified in the previous chapters and as such constitutes the overall discussion of the thesis.

8.2 Aim

To explore the relationship between dental factors, fluoride exposure and presence of a particular gene SNP and the occurrence of developmental defects of enamel and/or dental fluorosis.

8.3 Methods

8.3.1 Statistical plan

A statistical plan to inform a predictive model (Appendix BL) was developed. The variables of interest were identified on the basis of literature, clinical knowledge and the results of the 3 phases (Chapters 5 to 7). The variables were classified into continuous/categorical, dependent/independent and response/predictive variables and the predictive model was structured into 3 parts to capture the relevant variables.

8.3.2 Data handling

The Phase 1, 2 and 3 data entered into separate excel spreadsheet were checked for errors and omissions and exported to Statistical Package for Social Sciences (SPSS) Version 17, where the explanatory variables were recoded. .

8.3.3 Data analysis

Binary regression analysis was undertaken to estimate the relationship between the two dichotomous dependent variables (presence/absence of DDE; dental fluorosis) and the explanatory independent variables, at $p<0.05$. Linear regression was undertaken to predict the relationship between 2 continuous dependent variables (worst DDE score and worst TF score) and a number of independent variables at $p< 0.05$ (Appendix BL). Ordinal regression was also undertaken to predict the relationship between categorical variables and a number

of independent variables, at p <0.05. From the results of the regression analyses, binary regression was selected for the predictive modelling because the risk of occurrence of the defects could be generated using Exp(B), an Odds Ratio measure, R^2 values and the percentage of correct prediction. The Hosmer and Lemeshow goodness-of-fit test was undertaken to check if the data fit the model.

8.4 Results

The results are presented in Tables 8.1 to 8.10 based on the 3 Phases of the study, the age of the children and the dentition (primary and permanent). The p values of the Hosmer and Lemeshow goodness-of-fit test were not significant indicating that the data fit the model. A p value greater than 0.05 showed that the predictive model was satisfactory; i.e. there was no mis-specification of the predictive capacity of the model. The Nagelkerle R^2 from the predictive model showed the percentage variability in the dependent variable that was accounted for by the independent variables.

8.4.1 Phase 1 four year olds (n=302)

The Nagelkerle R^2 from the model was 0.075 and 0.090 for DDE and dental fluorosis respectively. This showed that 7.5% and 9% of the variability in the occurrence of DDE and dental fluorosis respectively was accounted for by the independent variables. After adjusting for other confounders at the 5% level, as Table 8.1 shows there were no statistically significant predictors of DDE (p>0.05) while Table 8.2 shows that the amount of toothpaste used per brushing and F toothpaste exposure were statistically significant predictors of dental fluorosis (p<0.05) in the primary teeth of 4 year olds with Odds Ratios of 9.66 (CI = 1.28-73.16) and 0.03 (CI = 0.02-0.70) respectively. For a one unit increase in the amount of F toothpaste used per brushing, the risk of having dental fluorosis was 9 times more while the odds of having dental fluorosis from 1 unit increase in F toothpaste exposure was low.

Predictors	Developmental dental defects (Yes/No) (R^2=0.075[1]; % Predicted =77.2%)				
	B	Sig (p)	Exp (B)	95% CI	
				Lower	Upper
Age (Years)	0.45	0.47	1.57	0.47	5.28
Gender (Male/Female)	-0.23	0.43	0.79	0.45	1.41
F Concentration Drinking Water (mg/l)	0.20	0.69	1.22	0.46	3.21
F Concentration Cooking Water (mg/l)	0.50	0.37	1.64	0.55	4.89
Exclusive Breast Feeding (No/Yes)	20.35	1.00	6.32b	0.00	[a]
Age of stopping Breast Feeding (Months)	20.35	1.00	6.81b	0.00	[a]
Infant/childhood disease (No/Yes)	0.45	0.13	1.57	0.88	2.81
Age of tooth brushing (Months)	0.11	0.72	1.12	0.61	2.07
Frequency of tooth brushing (1x, 2x & >2x)	0.33	0.56	1.39	0.46	4.26
Amount of toothpaste used per brushing (g)	0.61	0.23	1.85	0.68	5.00
Fluoride toothpaste exposure (µg/g)	-1.03	0.16	0.36	0.09	1.50
Normal birth (No/Yes)	0.78	0.32	1.18	0.48	10.0
Family history - tooth discolouration (No/Yes)	0.31	0.62	1.36	0.42	4.44

Table 8-1: Binary regression analysis model for DDE (Yes/No) in primary teeth of 4 year olds (n=302) for Phase 1 data. *Note: [1] – Nagelkerle R^2; [a] – Not reported because it is very negligible; b - x10^{-8}*

Predictors	Dental fluorosis (Yes/No) (R^2=0.090[1]; % Predicted =94.1%)				
	B	Sig (p)	Exp (B)	95% CI	
				Lower	Upper
Age (Years)	0.10	0.93	1.11	0.12	9.93
Gender (Male/Female)	0.15	0.79	1.16	0.41	3.27
F Concentration Drinking Water (mg/l)	-0.27	0.71	0.76	0.18	3.19
F Concentration Cooking Water (mg/l)	0.35	0.63	1.42	0.34	5.90
Exclusive Breast Feeding (No/Yes)	-18.20	1.00	0.00	0.00	[a]
Age of stopping Breast Feeding (Months)	-17.97	1.00	0.00	0.00	[a]
Infant/childhood disease (No/Yes)	-0.50	0.37	0.61	0.21	1.81
Age of tooth brushing (Months)	-0.19	0.74	0.83	0.28	2.49
Frequency of tooth brushing (1x, 2x & >2x)	0.97	0.29	2.64	0.44	15.91
Amount of toothpaste used per brushing (g)	2.27	**0.03[2]**	9.66	1.28	73.16
Fluoride Toothpaste exposure (µ/g)	3.39	**0.03[2]**	0.03	0.02	0.70
Normal birth (No/Yes)	-0.63	0.59	0.53	0.05	5.32
Family history - tooth discolouration (No/Yes)	0.61	0.48	1.83	0.35	9.73

Table 8-2: Binary regression analysis model for dental fluorosis (Yes/No) in primary teeth of 4 year olds (n=302) for Phase 1 data. *Note: [1] – Nagelkerle R^2; [2] – Statistically significant at P<0.05; [a] – Not reported because it is very negligible*

8.4.2 Phase 1 eight years primary dentition (n=322)

The Nagelkerle R^2 for the model was 0.045 and 0.22 for DDE and dental fluorosis respectively. This shows that 4.5% and 22% of the variability in the occurrence of DDE

and dental fluorosis respectively was accounted for by the independent variables. At the 5% level, as Table 8.3 shows there was no statistically significant predictor of DDE ($p>0.05$) while Table 8.4 shows gender, F concentration in drinking and cooking water, exclusive breastfeeding and frequency of tooth brushing were statistically significant predictors of dental fluorosis ($p<0.05$) in the primary teeth of 8 year olds with an Odds Ratio of 1.92 (CI=1.11-3.34), 1.85 (CI=1.04-3.26), 1.79 (0.97-3.30), 0.19 (CI=0.03-1.16) and 1.95 (CI=0.57-6.64) respectively. For 1 unit increase in the F concentration in drinking and cooking water and frequency of tooth cleaning, the risk of having dental fluorosis was about 2 times more compared to when there was no increase. Females had 1.92 times increased risk while presence of exclusive breastfeeding had 0.19 times decreased risk of dental fluorosis (Table 8.4).

Predictors	**Developmental dental defects (Yes/No) (R^2=0.045[1]; % Predicted =65.7%)**				
	B	**Sig (p)**	**Exp (B)**	**95% CI**	
				Lower	**Upper**
Age (Years)	-0.62	0.14	0.54	0.24	1.23
Gender (Male/Female)	-0.11	0.66	0.90	0.55	1.46
F Concentration Drinking Water (mg/l)	-0.18	0.50	0.83	0.50	1.40
F Concentration Cooking Water (mg/l)	-0.04	0.87	0.96	0.56	1.63
Exclusive Breast Feeding (No/Yes)	-0.48	0.54	0.62	0.14	2.86
Age of stopping Breast Feeding (Months)	-0.35	0.62	0.71	0.18	2.77
Infant/childhood disease (No/Yes)	0.37	0.13	1.45	0.89	2.36
Age of tooth brushing (Months)	-0.43	0.29	0.65	0.29	1.45
Frequency of tooth brushing (1x, 2x &>2x)	-0.16	0.79	0.85	0.26	2.77
Amount of toothpaste used per brushing (g)	-0.54	0.24	0.59	0.24	1.42
Fluoride Toothpaste exposure (µ/g)	0.69	0.34	1.99	0.49	8.12
Normal birth (No/Yes)	-1.20	0.28	0.30	0.03	2.65
Family history - tooth discolouration (No/Yes)	0.15	0.78	1.16	0.41	3.30

Table 8-3: Binary regression analysis model for DDE (Yes/No) in primary teeth of 8 year olds (n=322) for Phase 1 data. *Note: [1] – Nagelkerle R^2*

Predictors	Dental fluorosis (Yes/No) (R^2=0.22[1]; % Predicted =96.2%)				
	B	Sig (p)	Exp (B)	95% CI	
				Lower	Upper
Age (Years)	0.74	0.12	2.10	0.82	5.36
Gender (Male/Female)	0.66	**0.02[2]**	1.92	1.11	3.34
F Concentration Drinking Water (mg/l)	0.61	**0.03[2]**	1.85	1.04	3.26
F Concentration Cooking Water (mg/l)	0.58	**0.04[2]**	1.79	0.97	3.30
Exclusive Breast Feeding (No/Yes)	-1.65	**0.02[2]**	0.19	0.03	1.16
Age of stopping Breast Feeding (Months)	-0.24	0.73	0.79	0.20	3.09
Infant/childhood disease (No/Yes)	-0.05	0.99	1.00	0.58	1.72
Age of tooth brushing (Months)	0.78	0.14	2.17	0.77	6.14
Frequency of tooth brushing (1x, 2x &>2x)	0.67	**0.03[2]**	1.95	0.57	6.64
Amount of toothpaste used per brushing (g)	-0.28	0.57	0.76	0.29	1.96
Fluoride Toothpaste exposure (µ/g)	0.35	0.64	1.42	0.32	6.20
Normal birth (No/Yes)	-1.47	0.13	0.23	0.04	1.50
Family history - tooth discolouration (No/Yes)	-1.08	0.17	0.34	0.07	1.59

Table 8-4: Binary regression analysis model for dental fluorosis (Yes/No) in primary teeth of 8 year olds (n=322) for Phase 1 data. *Note: [1] – Nagelkerle R^2; [2] – Statistically significant at P<0.05.*

8.4.3 Phase 1 eight years permanent dentition (n=322)

The Nagelkerle R^2 value from the model was 0.13 and 0.14 for DDE and dental fluorosis respectively. This shows that 13% and 14% of the variability in the occurrence of DDE and dental fluorosis respectively was accounted for by the independent variables. As Table 8.5 shows, infant/childhood disease and F toothpaste exposure were statistically significant predictors of DDE with Odds Ratios of 2.13 (CI=1.27-3.57) and 4.44 (CI=0.98-20.09) respectively. The presence of infant/childhood disease and a 1 unit increase in F toothpaste exposure increased the risk of having DDE by 2.13 and 4.44 respectively. Table 8.6 shows that gender and birth conditions were statistically significant predictors of dental fluorosis in the permanent teeth of 8 year olds ($p<0.05$) with odds ratio of 0.50 (0.29-0.86) and 0.12 (0.02-0.66) respectively. Being female reduced the odds of occurrence of dental fluorosis to 0.5 ($p=0.01$), while having a normal birth also decreased the risk of dental fluorosis (Odds Ratio 0.12; $p=0.02$).

Predictors	Developmental enamel defects (Yes/No) (R^2=0.13[1]; % Predicted =67.6%)				
	B	Sig (p)	Exp (B)	95% CI	
				Lower	Upper
Age (Years)	-0.44	0.31	0.64	0.27	1.52
Gender (Male/Female)	-0.03	0.90	0.97	0.58	1.61
F Concentration Drinking Water (mg/l)	0.39	0.24	1.48	0.77	2.82
F Concentration Cooking Water (mg/l)	-0.20	0.52	0.82	0.44	1.52
Exclusive Breast Feeding (No/Yes)	0.99	0.40	2.69	0.27	26.69
Age of stopping Breast Feeding (Months)	1.88	0.08	6.54	0.78	54.65
Infant/childhood disease (No/Yes)	0.76	**0.01[2]**	2.13	1.27	3.57
Age of tooth brushing (Months)	-0.05	0.85	0.95	0.56	1.61
Frequency of tooth brushing (1x, 2x &>2x)	-0.43	0.50	0.65	0.19	2.49
Amount of toothpaste used per brushing (g)	-0.88	0.07	0.42	0.16	1.07
Fluoride Toothpaste exposure (µ/g)	1.49	**0.04[2]**	4.44	0.98	20.09
Normal birth (No/Yes)	-0.85	0.45	0.43	0.05	3.82
Family history - tooth discolouration (No/Yes)	-0.97	0.07	0.38	0.13	1.09

Table 8-5: Binary regression analysis model for DDE (Yes/No) in permanent teeth of 8 year olds (n=322) for Phase 1 data. *Note: [1] – Nagelkerle R^2; [2] – Statistically significant at P<0.05*

Predictors	Dental fluorosis (Yes/No) (R^2=0.14[1]; % Predicted =69.5%)				
	B	Sig (p)	Exp (B)	95% CI	
				Lower	Upper
Age (Years)	0.74	0.11	2.10	0.86	5.13
Gender (Male/Female)	0.69	**0.01[2]**	0.50	0.29	0.86
F Concentration Drinking Water (mg/l)	0.44	0.14	1.55	0.86	2.80
F Concentration Cooking Water (mg/l)	-0.42	0.20	0.66	0.35	1.25
Exclusive Breast Feeding (No/Yes)	1.22	0.14	3.40	0.67	17.31
Age of stopping Breast Feeding (Months)	0.24	0.72	1.27	0.34	4.68
Infant/childhood disease (No/Yes)	0.12	0.65	1.13	0.67	1.89
Age of tooth brushing (Months)	-0.06	0.82	0.94	0.55	1.60
Frequency of tooth brushing (1x, 2x &>2x)	0.30	0.62	1.34	0.42	4.32
Amount of toothpaste used per brushing (g)	-0.70	0.13	0.50	0.20	1.23
Fluoride Toothpaste exposure (µ/g)	0.81	0.27	2.24	0.54	9.23
Normal birth (No/Yes)	-2.15	**0.02[2]**	0.12	0.02	0.66
Family history - tooth discolouration (No/Yes)	-1.29	0.10	0.28	0.06	1.30

Table 8-6: Binary regression analysis model for dental fluorosis (Yes/No) in permanent teeth of 8 year olds (n=322) for Phase 1 data. *Note: [1] – Nagelkerle R^2; [2] – Statistically significant at P<0.05*

8.4.4 Phase 2 (n=125)

The Nagelkerle R^2 from the model was 0.29 and 0.46 for DDE and dental fluorosis respectively. This shows that 29% and 46% of the variability in the occurrence of DDE and dental fluorosis respectively was accounted for by the independent variables. Table 8.7 shows that at the 5% level, infant/childhood disease was the only statistically significant

predictor of DDE in the primary and permanent dentition of 4 and 8 year olds (p=0.01) with an Odds Ratio of 5.08 (CI=1.45-17.78). A history of childhood disease increased the risk of DDE by a factor of 5. Regarding TFI for measuring dental fluorosis, Table 8.8 shows that total daily F intake (TDFI) was the only statistically significant predictor of dental fluorosis (p=0.02) with an Odds Ratio of 5.41 (CI=1.39-21.02) in both primary and permanent teeth of the 125 study participants who contributed Phase 2 data. For a unit increase in TDFI, there was a 5 times increase in dental fluorosis risk.

Predictors	**Developmental enamel defects (Yes/No) (R^2=0.29[1]; % Predicted = 82.5%)**				
	B	**Sig (p)**	**Exp (B)**	**95% CI**	
				Lower	**Upper**
Age (Years)	0.49	0.09	1.64	0.93	2.88
Gender (Male/female)	0.60	0.29	1.81	0.60	5.46
F Concentration Drinking Water (mg/l)	1.43	0.41	4.18	0.14	129.23
F Concentration Cooking Water (mg/l)	-1.34	0.07	0.26	0.06	1.13
Total daily F intake (mg/day)	-0.56	0.31	0.57	0.19	1.69
Total daily F retention (mg/day)	0.95	0.13	2.60	0.77	8.79
Stunting (Normal/Abnormal)	0.04	0.38	1.04	0.95	1.14
Wasting/malnutrition (Normal/Abnormal)	0.02	0.44	1.02	0.98	1.06
Exclusive Breast Feeding (No/Yes)	20.43	1.00	7.26b	0.00	[a]
Age of stopping Breast Feeding (Months)	21.02	1.00	1.09b	0.00	[a]
Infant/childhood disease (No/Yes)	1.62	**0.01[2]**	5.08	1.45	17.78
Age of tooth brushing (Months)	0.21	0.75	1.24	0.33	4.70
Frequency of tooth brushing (1x, 2x &>2x)	0.24	0.83	1.27	0.15	11.03
Amount of toothpaste used per brushing (g)	0.72	0.47	2.05	0.29	14.51
Fluoride Toothpaste exposure (µg/g)	-1.71	0.24	0.18	0.01	3.07
Normal birth (No/Yes)	3.11	0.11	22.30	0.51	978.83
Family history - tooth discolouration (No/Yes)	0.15	0.88	1.16	0.17	7.68

Table 8-7: Binary regression analysis model for DDE (Yes/No) in the primary and permanent teeth of 4 and 8 year olds (n=125) for Phase 2 data. *Note: [1] – Nagelkerle R^2; [2] – Statistically significant at P<0.05; [a] – Not reported because it was negligible; [b] - x10^{-8}*

8.4.5 Phase 3 (n=70)

For those participants contributing Phase 1, 2 and 3 data (n=70), the Nagelkerle R^2 was 0.44 and 0.70 for DDE and dental fluorosis respectively indicating that 44% and 70% of the variability in the occurrence of DDE and dental fluorosis respectively was accounted for by the independent variables. Table 8.9 shows that cooking water F and total daily F retention were statistically significant predictors of DDE (p=0.04, OR=0.15 (CI=0.03-0.87)) and (p=0.04, OR=8.07 (CI=1.08-60.05)) respectively. Regarding dental fluorosis, Table 8.10 shows that total daily F intake (TDFI) and infant/childhood disease were statistically significant predictors of dental fluorosis (p=0.04, OR=11.83(CI=0.092-152.87)) and

(p=0.01, OR=58.79 (CI=2.55- 1355.52)) respectively, in the primary and permanent teeth of the 70 study participants. The risk of having dental fluorosis based on the presence of SNPs CC and AC was respectively 1.56(CI=0.62-20.32) and 3.85(CI=0.83-17.87) but their presence was not a statistically significant predictor (p>0.05).

Predictors	**Dental fluorosis (Yes/No) (R^2=0.46[1]; % Predicted = 88.3%)**				
	B	**Sig (p)**	**Exp (B)**	**95% CI**	
				Lower	**Upper**
Age (Years)	0.55	0.08	1.74	0.94	3.21
Gender (Male/female)	0.88	0.18	2.41	0.67	8.68
F Concentration Drinking Water (mg/l)	-0.36	0.54	0.70	0.22	2.19
F Concentration Cooking Water (mg/l)	0.22	0.79	1.25	024	6.43
Total daily F intake (mg/day)	1.69	**0.02[2]**	5.41	1.39	21.02
Total daily F retention (mg/day)	-1.39	0.07	0.25	0.06	1.13
Stunting (Normal/Abnormal)	-0.003	0.96	1.00	0.90	1.11
Wasting/malnutrition (Normal/Abnormal)	0.01	0.67	1.01	0.96	1.06
Exclusive Breast Feeding (No/Yes)	-19.60	1.00	0.00	0.00	[a]
Age of stopping Breast Feeding (Months)	-19.75	1.00	0.00	0.00	[a]
Infant/childhood disease (No/Yes)	1.30	0.07	3.68	0.90	15.10
Age of tooth brushing (Months)	-0.06	0.93	0.94	0.27	3.36
Frequency of tooth brushing (1x, 2x &>2x)	1.30	0.25	3.66	0.40	33.85
Amount of toothpaste used per brushing (g)	0.27	0.80	1.31	0.17	10.05
Fluoride Toothpaste exposure (µg/g)	-2.14	0.20	0.12	0.01	3.07
Normal birth (No/Yes)	-0.86	0.63	0.42	0.01	13.76
Family history - tooth discolouration (No/Yes)	-19.38	1.00	0.00	0.00	[a]

Table 8-8: Binary regression analysis model for dental fluorosis (Yes/No) in the primary and permanent dentition of 4 and 8 year olds (n=125) for Phase 2 data. *Note: [1] – Nagelkerle R^2; [2] – Statistically significant at P<0.05; [a] – Not reported because it is very negligible*

Predictors	Developmental enamel defects (Yes/No) (R^2=0.44[1]; % Predicted = 85.1%)				
	B	Sig (p)	Exp (B)	95% CI	
				Lower	Upper
Age (Years)	0.93	0.07	2.53	0.93	6.87
Gender (Male/female)	1.87	0.09	6.47	0.73	57.08
F Concentration Drinking Water (mg/l)	3.29	0.27	26.83	0.08	9.69[3]
F Concentration Cooking Water (mg/l)	1.91	**0.04[2]**	0.15	0.03	0.87
Total daily F intake (mg/day)	1.66	0.08	0.19	0.03	1.22
Total daily F retention (mg/day)	2.09	**0.04[2]**	8.07	1.08	60.05
Stunting (Normal/Abnormal)	0.05	0.57	1.05	0.88	1.26
Wasting/malnutrition (Normal/Abnormal)	0.06	0.21	1.06	0.97	1.16
Exclusive Breast Feeding (No/Yes)	21.47	1.00	2.01b	0.00	[a]
Age of stopping Breast Feeding (Months)	21.38	1.00	1.45b	0.00	[a]
Infant/childhood disease (No/Yes)	0.80	0.40	2.23	0.34	14.50
Age of tooth brushing (Months)	1.47	0.30	4.35	0.27	69.62
Frequency of tooth brushing (1x, 2x &>2x)	0.88	0.62	2.40	0.07	78.98
Amount of toothpaste used per brushing (g)	1.52	0.35	4.59	0.19	113.06
Fluoride Toothpaste exposure (µg/g)	-2.38	0.36	0.09	0.001	15.66
Normal birth (No/Yes)	2.54	0.37	12.72	0.05	3.94[3]
Family history - tooth discolouration (No/Yes)	-0.99	0.56	0.37	0.01	10.40
Single nucleotide polymorphism					
SNP CC	0.12	0.56	1.22	0.32	5.50
SNP AC	0.27	0.62	1.31	0.46	3.77

Table 8-9: Binary regression analysis model for DDE (Yes/No) in the primary and permanent dentition of 4 and 8 year olds (n=70) for Phase 3 data. *Note: [1] – Nagelkerle R^2; [2] – Statistically significant at P<0.05; [a] – Not reported because it is very negligible; [b] - $x10^{-10}$*

Predictors	Dental fluorosis (Yes/No) (R^2=0.70[1]; % Predicted = 88.1%)				
	B	Sig (p)	Exp (B)	95% CI	
				Lower	Upper
Age (Years)	0.57	0.31	1.77	0.59	5.37
Gender (Male/female)	1.45	0.23	4.26	0.40	45.14
F Concentration Drinking Water (mg/l)	-0.72	0.52	0.49	0.06	4.30
F Concentration Cooking Water (mg/l)	-2.25	0.42	0.11	0.00	25.47
Total daily F intake (mg/day)	2.47	**0.04[2]**	11.83	0.92	152.87
Total daily F retention (mg/day)	-1.47	0.27	0.23	0.02	3.16
Stunting (Normal/Abnormal)	-0.06	0.59	0.94	0.76	1.17
Wasting/malnutrition (Normal/Abnormal)	0.06	0.24	1.06	0.96	1.16
Exclusive Breast Feeding (No/Yes))	-10.41	1.00	0.00	0.00	[a]
Age of stopping Breast Feeding (Months)	-12.82	1.00	0.00	0.00	[a]
Infant/childhood disease (No/Yes)	4.07	**0.01[2]**	58.79	2.55	1355.52
Age of tooth brushing (Months)	2.22	0.17	9.19	0.38	220.43
Frequency of tooth brushing (1x, 2x &>2x)	-0.61	0.73	0.55	0.02	17.60
Amount of toothpaste used per brushing (g)	-1.51	0.42	0.22	0.01	8.69
Fluoride Toothpaste exposure (µg/g)	-3.39	0.40	0.03	0.00	87.45
Normal birth (No/Yes)	-2.89	0.28	0.06	0.00	11.02
Family history - tooth discolouration (No/Yes)	-14.63	1.00	0.00	0.00	[a]
Single nucleotide polymorphism					
SNP CC	1.10	**0.08**	1.56	0.62	20.32
SNP AC	1.35	**0.08**	3.85	0.83	17.87

Table 8-10: Binary regression analysis model for dental fluorosis (Yes/No) in the primary and permanent dentition of 4 and 8 year olds (n=70) for Phase 3 data. *Note: [1] – Nagelkerle R^2; [2] – Statistically significant at P<0.05; [a] – Not reported because it is very negligible*

8.4.6 Summary of results

8.4.6.1 Developmental Defects of Enamel

For the permanent teeth of 8 year olds (Phase 1 data; n=322):

- F toothpaste exposure (μ/g) (p=0.04) and a history of infant/childhood disease (p=0.01) were significant predictors of DDE.

For the primary and permanent teeth of 4 and 8 year olds (Phase 2 data; n=125):

- A history of infant/childhood disease was a statistically significant predictor of DDE (p=0.01).

For the primary and permanent teeth of 4 and 8 year olds (Phase 3 data; n=70):

- The risk of DDE increased as the cooking water F concentration (p=0.04) and Total Daily Retention (p=0.04) increased.

8.4.6.2 Dental Fluorosis

For the primary teeth of 4 year olds (Phase 1 data; n=302):

- The amount of toothpaste used per brushing (g) (p=0.03) and F toothpaste exposure (μ/g) (p=0.03) were statistically significant predictors of dental fluorosis.

For the primary teeth of 8 year olds (Phase 1 data; n=322):

- The risk of females having dental fluorosis was 1.92 times greater than for males (p=0.02).
- The risk of having dental fluorosis increased as the drinking water F (p=0.03), cooking water F ((p=0.04) and tooth brushing frequency (p=0.03) increased.

For the permanent teeth of 8 year olds (Phase 1 data; n=322):

- The risk of females having dental fluorosis was 0.5 times the risk for males (p=0.01).
- The risk of those whose birth was normal having dental fluorosis was 0.12 times the risk for those having had an abnormal birth (p=0.02).

For the primary and permanent dentition of 4 and 8 year olds (Phase 2 and 3 data)

- Total daily F intake was a statistically significant predictor (p=0.02) for dental fluorosis (Phase 2 data; n=125).

- Total daily F intake (p=0.04) and a history of infant/childhood disease (p=0.01) were statistically significant predictors for dental fluorosis (Phase 3 data; n=70):
- Presence of SNPs CC (p=0.08) and AC (p=0.08) of COL1A2 had a tendency but failed to be the statistically significant predictors of dental fluorosis in both primary and permanent teeth of 4 and 8 year olds (Phase 3 data; n=70).

8.5 Discussion

8.5.1 Overall aim

The aetiology of developmental defects of enamel is not well understood, although the main risk factors are generally accepted to be environmental. However, there have been several indications of a potential influence of genetic factors on susceptibility to enamel defects especially dental fluorosis. Some studies (Huang et al., 2008, Wang et al., 2010, Ba et al., 2011, Wen et al., 2012) have reported results of a candidate gene approach providing support for the plausibility of a role of genetic factors in dental fluorosis aetiology. The promising findings of these studies were based on a design strategy that targeted communities with both high and low F concentrations in drinking water.

The associations between the candidate genes and dental fluorosis were seen only within the high-F community samples and in fact, all identified cases of dental fluorosis came from these communities. This is suggestive of gene-environment interaction in the occurrence of dental fluorosis, however, a possible analytical approach to formally evaluate the interaction would be a regression model incorporating the effects of genotype, dental factors and F exposure as well as other influencing factors. Therefore, this present PhD project investigated the influence of F exposure (intake and excretion), genetics, dental factors as well as other environmental factors on the occurrence of developmental defects of enamel and dental fluorosis among 4 and 8 year olds in Nigeria through a cross-sectional observational survey. Findings from this project provide information that should help influence policy and practice concerning the prevention of risk factors for DDE and dental fluorosis as well as excessive F exposure at individual and community level.

8.5.2 Overall method

A pilot study of F concentration of water from common sources in the study locations was undertaken to categorise the locations prior to main study. The main study was carried out in 3 phases, comprising anthropometric measurements, clinical dental examination to determine the occurrence of DDE and dental fluorosis, laboratory measurement of F

concentration to estimate F exposure and genetic analysis to determine presence of SNP in COL1A2 genes. The overall method involved the development of regression models for each phase of the study as more explanatory variables were successively added to predict the occurrence of developmental defects of enamel and dental fluorosis from a number of environmental and genetic factors. Previous studies (Huang et al., 2008, Ba et al., 2011) on gene-environment interaction in the aetiology of dental fluorosis only included drinking water F concentration leaving out other environmental factors reported in the aetiology of dental fluorosis such as malnutrition, breastfeeding and infant or childhood diseases and Odds Ratios were generated after adjusting for only age and gender. In this present study, the regression models generated Odds Ratios after adjusting for age, gender and other influential explanatory variables such as drinking and cooking water F, total daily F intake, total daily F retention, stunting, wasting/malnutrition, exclusive breastfeeding, age of stopping breastfeeding, infant/childhood diseases, age of tooth brushing, frequency of tooth brushing, amount of toothpaste used per brushing, F toothpaste exposure, normal birth and family history of tooth discolouration.

One of the limitations of this study was information bias from respondent or memory bias since parents/legal guardians were interviewed on past events. Also, achieving a dry field when clinically examining participants' mouths for presence of defects was very difficult for some study participants who salivated profusely. It was also very challenging to maintain dry tooth surfaces when images of anterior teeth of these children were taken. Collecting drink and food samples from parents/legal guardians of study participants was also challenging; some parents/legal guardians were unable to provide complete samples from their children. Transporting food and drink samples to the UK for F analysis was challenging because of activities of Nigerian and UK customs at the airports which caused about 4 to 5 hours delay. This delay led to samples starting to defrost before they finally arrived at the F Research laboratory, Newcastle University. The food and drink samples were immediately transferred to the - 20 ^{O}C freezer since they have not started to perish. The buccal mucosal swabs were stored at 4^{O}C in a fridge at the laboratory until when they were analysed and this did not affect the buccal mucosa samples since RNAlater, the storage media doesn't actually need to be frozen.

8.5.3 Overall results in context

The findings from the pilot study showed that the water F concentration from common sources in the study locations varied widely between area from 0.04-3.0 ppmF and within

area; for example in Area 2 it ranged from 0.07-3.0ppmF. As a result, area could not be included as a possible predictor for DDE or dental fluorosis and drinking water and cooking water F were used instead. The risk factors for developmental enamel defects may be attributed to local, systemic, genetic and environmental factors, but most are likely to be multifactorial in nature. The multifactorial nature of the risk factors of developmental enamel defects was observed in these study locations where the mouth (77.4%) and mean(SD) tooth prevalence (4.0(3.33) of DDE in primary teeth of 4 year olds was 77.4% compared to permanent teeth of 8 year olds (64.6% and 2.03(1.91) respectively). Conversely, the corresponding figure for dental fluorosis in primary teeth of 4 year olds was lower (5.6% and 0.24(1.26) respectively) compared to permanent teeth of 8 year olds (29.8% and 2.09(1.26) respectively) when TFI was used as index of measurement. Higher prevalence of maternal illnesses during pregnancy which affect enamel formation in developing primary tooth buds could be the reason why the prevalence of DDE in primary teeth of 4 year-olds was higher than in permanent teeth of 8 year-olds. The reason for the higher prevalence of dental fluorosis in the permanent teeth of 8 year-olds compared to primary teeth of 4 year-olds might be due to the influence of environment factors such as F which postnatally affect permanent tooth buds while the placenta reduces the transport of F to primary tooth buds *in-utero*.

For 4 and 8 year-olds, the prevalence of both DDE and dental fluorosis was higher in rural area (Area 2) with a higher mean F concentration in drinking (1.10ppmF for 4 year olds, 1.11ppmF for 8 year olds) and cooking (1.10ppmF for 4 year olds, 1.16ppmF for 8 year olds) water compared to rural area (Area 4) with lower mean F concentration in drinking (0.25ppmF for 4 year olds, 0.27ppmF for 8 year olds) and cooking (0.29ppmF for 4 year olds, 0.27ppmF for 8 year olds). This demonstrates the influence of water F used in drinking and cooking on the occurrence of dental fluorosis. In contrast, the prevalence of the 2 types of defects was lower in urban area (Area 3) with higher mean F concentration in drinking (0.53ppmF for 4 year olds, 0.75ppmF for 8 year olds) and cooking (0.31 ppmF for 4 year olds, 0.56ppmF for 8 year olds) water compared to urban area (Area 1) with lower mean F concentration in drinking (0.35ppmF for 4 year olds, 0.25ppmF for 8 year olds) and cooking (0.35ppmF for 4 year olds, 0.24ppmF for 8 year olds) water. Study participants in urban Area 1 might have genes that influence the occurrence of these defects. Based on TFI, the prevalence of dental fluorosis among children who drank or consumed diets cooked with low, moderate and high F water ranged between 4.3% - 8.0% in primary teeth of 4 year olds while it was 24.5% - 77.8% for permanent teeth of 8 year olds. The higher

prevalence of dental fluorosis in permanent teeth compared to primary teeth demonstrated the impact of longer periods of exposure of permanent tooth buds to water F and the *in-utero* prevention of the transport of F to primary tooth buds by the placenta. When regression analysis was undertaken to estimate the relationship between the two dichotomous dependent variables (presence/absence of dental fluorosis) and explanatory independent variables, dental fluorosis presence was related to drinking water F and cooking water F for the primary teeth of 8 year olds based on the Phase 1 data (n=322) and to the cooking water F for the primary and permanent dentition of the 4 and 8 year olds based on the Phase 3 data (n=70). These observed differences might be from the smaller sample size of Phase 3 which also include a mix of 4 and 8 year olds. The data may not be big enough to show true differences because it is a weaker source of data.

Some parents/legal guardians reported that their child/ward were not exclusively breastfed until weaning and the regression analysis showed that 8 year-olds who were not exclusively breastfed had increased risk of dental fluorosis in their primary dentition compared to those who were. These present findings concur with previous reports of an association between children who did not breastfeed (Kumar et al., 1998, Lunardelli and Peres, 2006) and the occurrence of enamel defects in primary teeth. This might be because breast milk contain very low amount of F (<0.01 – 0.019 ppmF) (Koparal et al., 2000, Sener et al., 2007)and a child who is not exclusively breastfed is at risk of F exposure from other fluids such as infant formulas made with water. In addition, reduced or lack of breastfeeding especially exclusive breastfeeding predisposes children to illnesses that might increase the risk of developing tooth buds to environmental factors that will cause enamel defects including dental fluorosis. In accordance with findings from other studies (Evans, 1991, Pendrys, 2000, Martins et al., 2011c), in the Phase 1 aspect of the regression model, it was surprising to observe that increased toothpaste used per brushing and F toothpaste exposure (amount of toothpaste used and frequency of brushing) were statistically significant predictors of dental fluorosis in primary teeth of the 302 4-year-olds. The calcification of the primary tooth buds would be largely complete before the 4 year-olds start using toothpaste (which is usually around the age of 6 months when the first primary teeth start to erupt), therefore F ingestion from toothpaste would be less likely to have an effect on their primary teeth. The present study also found an association between increase in frequency of tooth brushing and dental fluorosis in the primary teeth of 8 year-olds; a finding that has also been reported in other studies (Pendrys et al., 1994, Vallejos-Sanchez et al., 2006, Martins et al., 2011b). Conversely, in yet other studies, the association between toothpaste use and dental fluorosis

has not been statistically significant (Holm and Andersson, 1982, Riordan and Banks, 1991), especially in the primary dentition. Similarly, no significant association between frequency of tooth brushing and dental fluorosis was found when a meta-analysis of four cross-sectional surveys was performed Wong et al. (2010). In addition, Warren et al. (2001) in a study on dental fluorosis in primary dentition reported no significant association between dental fluorosis and use of toothpaste. The odds of DDE occurring in permanent teeth of 8 year olds increased as the F toothpaste exposure increased which might be due to the diffuse opacity component of the DDE. Those who were exposed to higher F toothpaste had 4 times risk of developing DDE in their permanent teeth. This was most likely due to the contribution of toothpaste to total daily F intake since toothpaste was the second item contributing most to TDFI, at 25% to 29%, often due to twice or more tooth brushing reported by parents/legal guardians of some participants as well as use of more than 2.5g toothpaste per brushing by the majority of the participants.

In the both primary and permanent teeth of 8 year olds, the risk of developing dental fluorosis was higher in females when compared to males and this finding is supported by some studies (Ramezani et al., 2004, Bardal et al., 2005, Rigo et al., 2010). Based on TFI, there was a higher prevalence of dental fluorosis among in the primary teeth of 4-year (3.0%) and permanent teeth of 8-year (18.6%) old females compared to 4-year (2.6%) and 8-year (10.6%) males. On the other hand, some studies (Mann et al., 1990, Warren et al., 1999, Narwaria and Saksena, 2013) have reported that the risk is higher in males than females while some studies (Ruan et al., 2005a, Zhang et al., 2014a) reported no gender difference. Different genetic makeup which may be linked to the sex chromosomes and method of assessing dental fluorosis in the different populations investigated might be the reason for these observed differences. The influence of gender on risk remains unclear currently and although it would be interesting to investigate this aspect further, it is unlikely to be clinically relevant in terms of managing the problem in at risk populations. There is uncertainty around genetic aspects of fluorosis and as such not much is known. In accordance with findings from several studies (Kumar and Swango, 1999, Warren et al., 2001, Molina-Frechero et al., 2012) in the current study the risk of fluorosis increased as the water F concentration increased with a weak to moderate relationship between severity of dental fluorosis and F concentration in drinking and cooking water observed in Chapter 5.

Although parents or legal guardians of study participants were not asked about previous history of malarial infection, an endemic tropical disease, the occurrence of infectious

diseases which sometimes present like malaria or occur together with malaria during infancy or childhood among study participants was reported by some parents/legal guardians. Infectious diseases caused by bacteria and viruses such as chicken pox, rubella, measles, mumps and influenza have been associated with DDE in both primary and permanent teeth (Seow, 1991, Ford et al., 2009). Consistently with other studies (Arrow, 2009, Guergolette et al., 2009), diseases during infancy or childhood were associated with the occurrence of DDE and dental fluorosis in this present study. DDE and dental fluorosis in the both primary and permanent teeth of 4 and 8 year olds in Phases 2 and 3 of the study were associated with infant/childhood disease because fever and derangement of acid-base balance from infections is known to directly damage ameloblasts as well as the developing enamel prisms formed during mineralisation (Seow, 1991). It was subsequently observed that children whose births had not been normal were significantly more likely to develop dental fluorosis in their permanent teeth due to postnatal alteration in calcium homeostasis and the stress placed on developing tooth buds. A previous study in Senegal (Diouf et al., 2012) reported an association between low birth weight babies and dental fluorosis but this was at variance with another study in New York, USA (Kumar and Swango, 2000) that did not report any association. The differences in the reported association between birth conditions and dental fluorosis might be due to differences in aetiological factors of low birth weight and methods used to assessing dental fluorosis.

The prevalence of dental fluorosis has been reported as increasing among populations consuming non-fluoridated as well as fluoridated water (Diesendorf, 2003, Khan et al., 2005). Much of the increased prevalence of dental fluorosis in non-fluoridated and optimally fluoridated communities is due to widespread use of F from sources other than drinking water such as beverages, foods and toothpaste (Riordan, 2002, Cury and Tenuta, 2014). In this present study, food was the primary contributor to total daily F intake and when anthropometrics, total daily F intake and retention data from Phase 2 were included into the regression model, total daily F intake was the only significant predictor of dental fluorosis in the teeth of both primary and permanent teeth of 4 and 8 year olds. Total daily F intake was also a significant predictor of dental fluorosis in both primary and permanent teeth of 4 and 8 year olds when the Phase 3 SNP data were included into the regression model. The odds of having dental fluorosis was 12 times when the total daily F intake increase. The association between TDFI and dental fluorosis might be due to the higher mean values for TDFI observed in both 4 (0.14 mg/kg bw/day and 8 (0.11 mg/kg bw/day) year-olds when compared to the optimal range of 0.05-0.07 mg/kg bw/day. The positive

relationship between dental fluorosis and total daily F intake is in agreement with a study (Molina-Frechero et al., 2012) on risk factors of dental fluorosis among Mexican children where F exposure was associated with dental fluorosis. Other studies (Fomon et al., 2000, Erdal and Buchanan, 2005) have also shown that a F intake increase can be associated with further increase in dental fluorosis prevalence.

Furthermore, those who retained more F daily were more likely to develop DDE in both primary and permanent teeth. The risk of developing DDE when total daily F retention increased by one unit had an approximately 8 times higher risk. For 4 and 8 year-olds in Area 2, the mean (SD) TDFI was respectively 0.39(0.19) mg/kg bw/day and 0.33(0.12) mg/kg bw/day higher than optimal range of F (0.05-0.07) mg/kg bw/day and as TDFI increased, the F retention also increased. This might be the reason for the observed higher prevalence of DDE and dental fluorosis in Area 2, rural Igboora with higher drinking and cooking water F. According to chapter 6, there was a positive correlation between TDFI and FFR at a TDFI lower than 2.5 mgF/day and 3.0 mgF/day for 4 and 8 year-olds respectively. But for TDFI higher than these values (2.5 mgF/day and 3.0 mgF/day), FFR tended to reach limiting constant values independently of how high the TDFI was.

Regarding genetic influences on dental fluorosis, previous studies (Huang et al., 2008, Ba et al., 2011) have reported an association between polymorphisms in the COL1A2 gene with dental fluorosis in high F exposed populations after adjustment of age and gender. Homozygous CC genotype of COL1A2 gene was the risk genotype for dental fluorosis among children who lived in endemic fluorosis and high F concentration areas (Huang et al., 2008). Conversely, in this present study, children who were carriers of SNPs CC and AC in COL1A2 gene were respectively 1.56 and 3.85 times more likely to develop dental fluorosis ($p=0.08$) although this potential predictor failed to reach statistical significance. The small sample size in Phase 3 of this study and inclusion of several influential environmental factors as well as age and gender might be the reason why this potential predictor did not reach statistical significance. The presence of SNP AC resulted in a higher risk of having dental fluorosis compared to presence of SNP CC and might be due to the higher proportion of study participants with the heterozygote SNP AC genotype of COL1A2. Few children had SNP CC genotype and the majority of children who had dental fluorosis were carriers of C allele (either as CC or AC), therefore, it seems that C allele in SNPs AC or CC might be the risk allele. Further research in a larger population and in different study populations would be useful to confirm or dispute this finding.

8.6 Implications of the overall findings

- DDE and dental fluorosis were relatively common oral health problems observed among 4 and 8 year olds in the study locations which could result in aesthetics problems, tooth sensitivity, tooth wear and dental caries thereby affecting the quality of life of these children.
- These enamel defects might be caused by the following reported perinatal and postnatal factors: abnormal birth, infant/childhood diseases, lack of exclusive breastfeeding, brushing teeth more than twice daily and use of > 2.5 g toothpaste per brushing.
- The high concentration of F in some drinking and cooking water samples in this study was a prime reason for high intake of F in some areas. Foods, especially soups and rice and beans dishes cooked with water were the largest contributors to total daily F intake There was an association between prevalence of dental fluorosis and F concentration in water and a weak to moderate relationship between severity of dental fluorosis and F exposure in drinking and cooking water. If adequate measures are not instituted to provide optimal F concentration in water in areas where this is currently high, the prevalence of fluorosis will continue to be an oral and general health problem for communities.
- In Area 2, a rural area, the TDFI was 0.39 mg/kg bw/day and 0.33 mg/kg bw/day for 4 and 8 year-olds respectively: higher than the optimal range (0.05-0.07 mg/kg bw/day) which translated into a higher prevalence of dental fluorosis in this Area.
- The measured F concentration of toothpastes was lower than the labelled F concentration (ppm or %) on the toothpaste tube/packaging. Non specification of F concentration (ppm or %) makes it difficult for consumers to make an informed choice of the amount of F content in the toothpastes. Two toothpastes recorded a F concentration lower than 500 ppm which is not enough for the prevention of dental caries especially among high risk children.
- Environmental factors such as increased amounts of toothpaste used per brushing, higher F concentrations in drinking and cooking water, lack of exclusive breastfeeding, higher frequencies of tooth brushing, abnormal birth, higher total daily F intakes and F retention as well as a history of infant/childhood diseases were predictors of DDE and dental fluorosis, therefore efforts should be made to prevent their occurrence.

- Presence of the SNPs CC and AC of COL1A2 showed a tendency towards being statistically significant predictors of dental fluorosis. The C allele was of particular interest as a possible risk allele for dental fluorosis. It may therefore be a useful genetic marker for the differential risk of dental fluorosis but this area needs further research.

8.7 Recommendations

8.7.1 Dentistry

- Efforts should be made by dental practitioners to prevent prenatal, perinatal and postnatal causes of enamel defects, including dental fluorosis, through oral health promotion to women especially pregnant women and nursing mothers.
- Toothpastes contribute about one quarter to one third to total daily F intake for both 4 and 8 year olds which may be injurious to the developing tooth bud. Therefore, parents/guardians should be educated by dental practitioners to control the ingestion of F from toothpaste by young children especially when the toothpastes contain high concentration of F.
- Parents/guardians should be encouraged through dental hygiene programmes to brush the teeth of their children/wards twice daily with F toothpaste and to limit the amount of toothpaste used at each brushing.
- Dentists practicing in Area 2 with higher F concentration in water and prevalence of dental fluorosis in 4 and 8 year-olds should emphasise the need to limit F ingestion and be cautious in providing F therapy to children living in this area if the need arises.

8.7.2 Nutritionists

- Nutritionist should organise nutritional programmes for parents/guardians to encourage them to try, where possible and without compromising the child's dietary balance, to reduce their child/ward's consumption of particularly high F containing food items and increase their consumption of other lower F food items especially during the critical period of tooth development.
- Parents should be encouraged to practice exclusive breastfeeding and should breastfeed their child for longer duration.

8.7.3 Ministry of Health

- The Ministry of Health has the responsibility to ensure that people live healthily, therefore the Ministry's role is very important in the control and prevention of developmental defects of enamel and dental fluorosis. Efforts should be made to provide dental health education about the prevention of enamel defects, including dental fluorosis to women especially pregnant women and nursing mothers.
- Geographical mapping of water F concentrations in community water supplies would be helpful to identify those with high F concentrations and provide substitute with other sources of water supply or reduce the F concentration of water by defluoridation. However, considering the cost and availability of equipment for defluoridation of water as well as training personnel for plant maintenance, provision of optimum F concentration water for drinking through sachets might be a reasonable alternative approach.
- Nutritional and dental hygiene programmes delivered through the media or schools in communities where the prevalence of developmental defects of enamel and dental fluorosis is high relative to other areas to improve families' knowledge of these enamel defects.
- Funding for further genetic studies on the influence of genes on the occurrence of enamel defects, including dental fluorosis, in larger population groups could help determine the role of genes in these conditions.

8.7.4 Toothpaste manufacturers

- Toothpaste manufacturers should undertake regular quality control of F concentration in toothpaste.
- Clear labelling of the F concentration of toothpaste using the widely accepted unit of ppmF should be undertaken.
- Toothpastes should be labelled with the recommendation that parents/ guardians should supervise brushing of the teeth of their children/wards at least before the age of 7 years and that the amount of toothpaste used should be limited to a small pea size.

Chapter 9 Overall conclusions

9.1 Introduction

This chapter presents an overall conclusions and recommendations for future work.

9.2 Overall conclusions

1) The mouth and mean(SD) tooth (number of teeth with defect) prevalence of DDE in the primary teeth of 4 year olds was respectively 77.8% and 4.0(3.33) while the corresponding figures for dental fluorosis (TFI) were respectively 5.6% and 0.24(1.26).
2) The mean(SD) mouth and tooth prevalence of developmental defects of enamel in the primary teeth of 8 year olds were respectively 64.9% and 2.03(1.91) while the corresponding figures for dental fluorosis (TFI) were respectively 9.3% and 0.45(1.66).
3) The mean(SD) mouth and tooth prevalence of developmental defects of enamel in the permanent teeth of 8 year olds were respectively 67.1% and 2.37(2.22) while the corresponding figures for dental fluorosis (TFI) were respectively 29.8% and 2.09(3.66).
4) Based on TFI, the prevalence of dental fluorosis in primary teeth was 5.9%, 4.3% and 6.3% among 4 year old participants who drank low, moderate and high F concentration water while it was 5.5%, 8.0% and 6.7% for children who consumed diets cooked with low, moderate and high F water. There was a weak relationship between the prevalence and severity of dental fluorosis in primary teeth and F exposure in drinking water among 4 year olds (ρ=0.12; p=0.04).
5) Based on TFI, the prevalence of dental fluorosis in primary teeth was 8.5%, 7.1% and 22.1% among 8 year old participants who drank low, moderate and high F concentration water while it was 8.5%, 10.0% and 22.0% respectively in children who consumed diets cooked with low, moderate and high F water. There was a weak relationship between the severity of dental fluorosis in primary teeth and F exposure in drinking water among 8 year olds (ρ=0.15; p=0.01).
6) Based on TFI, the prevalence of dental fluorosis in permanent teeth was 24.7%, 64.3% and 59.1% among 8 year old participants who drank low, moderate and high F concentration water while it was 24.5%, 45.0% and 77.8% respectively in children who consumed diets cooked with low, moderate and high F water. There was a moderate relationship between the severity of dental fluorosis in permanent

teeth and F exposure in drinking (ρ=0.28; p=0.001) and cooking (ρ=0.17; p=0.002) water among 8 year olds.

7) The relative contribution of drink, food and toothpaste to total daily F intake was respectively 17%, 54% and 29% for 4 year-olds while the corresponding values for 8 year-olds were 21%, 54% and 25%.
8) The relationship between total daily F intake (mg/day) and urinary F excretion (mg/day) was linear, moderate and strong for both 4- (ρ=0.41; p=0.001) and 8- (ρ=0.57; p<0.001) year-olds.
9) The relationship between TDFI and fractional F retention was linear, moderate and strong for both 4 (ρ=0.56; p<0.001) and 8 (ρ=0.50; p<0.001) year-olds at a TDFI of approximately 2.5 mgF/day and 3.0 mgF/day respectively and higher than these values the estimated FFR tends to reach limiting constant values independent of how high the TDFI is.
10) There was no statistical significant relationship between nutritional deficiency and F excretion and retention variables (p>0.05).
11) Fluoride toothpaste use, gender, drinking water F, cooking water F, lack of exclusive breastfeeding, infant/childhood diseases, TDFI and TDFR were statistically significant predictors of DDE and dental fluorosis (p<0.05).
12) Although only found to have a tendency towards statistical significance (p=0.08) in this study, a COL1A2 single nucleotide polymorphism may potentially be a useful marker for estimating the differential risk of dental fluorosis.

9.2.1 Recommendations for future work

- There is dearth of studies on the epidemiology of developmental enamel defects in primary teeth, especially in Africa, therefore, further research should be undertaken among children living in different water F concentration areas. Collaborative research in other parts of Nigeria and Africa most especially in the Northern part of Tanzania where the F concentration in water and the prevalence of dental fluorosis is very high would help determine risk of dental and skeletal fluorosis so that preventive strategies to mitigate risk could be introduced.
- Further epidemiological work on enamel defects, including dental fluorosis, to include other elements such as relationship with dental caries and dose-response relationship with F concentration in water should be undertaken among children. These findings would show how the epidemiological profile changes over time.

- Actual F concentration of some drink and food items consumed by study participants could not be determined because samples were not available. Therefore, further research is required to provide information on F concentration on commonly consumed drink and food items in Nigeria.
- Further research should be undertaken in different parts of Nigeria to inform policy and practice. By using the information on the F content of different Nigerian drinks and foods, further research on F intake in Nigeria will be easier.
- This is the first fluoride intake and excretion (FIE) study undertaken in Nigeria. Further FIE studies in other parts of Nigeria and Africa would help determine risk of dental and skeletal fluorosis so that preventive strategies to mitigate risk could be introduced.
- Considering the limitation of the genetic part of this study, it would be useful to replicate or confirm these findings in different populations with larger sample sizes while gathering detailed information from dental examination such as number of teeth with defects, concurrently. Dental fluorosis is a complex condition and it is likely that other genes may influence its occurrence, therefore, further investigation on other polymorphisms and other candidate genes would be an important area for further work.

Appendix A – Keywords that were used in the search strategy.

Concept 1 - Developmental	**Concept 2 – Defects**	**Concept 3 – Dental**	**Concept 4 – Fluorosis**	**Concept 5 - F**	**Concept 6 - Indices**	**Concept 7 – 4 and 8 years**	**Concept 8 – Genetics**	**Concept 9 – Urinary**	**Concept 10 - Diets**	**Concept 11 - Photograph**
Developmental	Defects	Dental	Fluorosis	Fluoride	Indices	4 years	Genetics	Urinary	Diets	Photograph
Hereditary	Malformation	Enamel	Mottling	Fluorine	Index	8 years	Genes	Excretion	Nutrition	Images
Formation	Anomaly	Tooth	Mottled		Diagnosis	4 and 8 years	Genome	Discharge	Foods	Pictures
Maturation	Aberration	Teeth	Discolouration		Devices	4 to 8 years	Genomic		Drinks	
Growth	Disturbance	ameloblasts			Tools	Children	Nucleotide		Intake	
Inheritance	Alteration				Measurement	School age	Polymorphism		ingestion	
	Abnormalities					Primary school	DNA		water	
	Opacities					Nursery	RNA			
	Hypoplasia									
	Discolouration									

Appendix B – MEDLINE via OVID Search Strategy.

Search strategy 1 – Developmental/Defects/Dental/Fluorosis/Indices/4 years/8 years

1. Develop* or grow* or form* or matur* or inherit* or hereditary
2. Defect* or malfor* or anomal* or aberration* or disturb* or alterat* or abnormal* or opacit* or hypoplasia* or discolour*
3. Dental or enamel or tooth or teeth or ameloblast*
4. Fluoros* or mottl* or discolour*
5. Ind* or diagnos* or device* or tool*
6. 4 year* or 8 year* or 4 to 8 year* or 4-8 year* or child* or school child* or school age*
7. 1 and 2 and 3 and 4 and 5 and 6

Search strategy 2 – F/Diets/4 years/8years

1. F/exp or fluori*
2. Diet* or nutri* or food* or drink* or intake* or ingest* or water
3. 4 year* or 8 year* or 4 to 8 year* or 4-8 year* or child* or school child* or school age*
4. 1 and 2 and 3

Search strategy 3 – F/Urinary/4 years/8 years

1. F/exp or fluori*
2. Urin* or excret* or discharge*
3. 4 year* or 8 year* or 4 to 8 year* or 4-8 year* or child* or school child* or school age*

Search strategy 4 – Developmental /Defects/Dental/Fluorosis/Genetics

1. Develop* or grow* or form* or matur* or inherit* or hereditary
2. Defect* or malfor* or anomal* or aberration* or disturb* or alterat* or abnormal* or opacit* or hypoplasia* or discolour*
3. Dental or enamel or tooth or teeth or ameloblast*
4. Fluoros* or mottl* or discolour*
5. Gen* or nucleotide* or polymorph* or DNA or RNA
6. 1 and 2 and 3 and 4 and 5

Search strategy 5 - Developmental /Defects/Dental/Fluorosis/Photograph

1. Develop* or grow* or form* or matur* or inherit* or hereditary
2. Defect* or malfor* or anomal* or aberration* or disturb* or alterat* or abnormal* or opacit* or hypoplasia* or discolour*
3. Dental or enamel or tooth or teeth or ameloblast*
4. Photo* or image* or picture*
5. 1 and 2 and 3 and 4.

Appendix C: Mean (±SD) fluoride concentration (mg/L) of water samples collected from wells and boreholes in the wards of the 2 LGAs: Ibadan North (Urban) and Ibarapa Central (Rural) LGAs (Stage 1 of Pilot Study).

Ward No.	Ibadan North LGA				Mean (SD) (mg/L)	Ibarapa Central LGA				Mean (SD) (mg/L)
	Area 1		Area 2			Area 1		Area 2		
	Water source	Fconc (mg/L)	Water source	F conc (mg/L)		Water source	F conc (mg/L)	Water source	F conc (mg/L)	
1	Well	0.10	Borehole	0.20	0.15 (0.07)	Well	0.30	Borehole	2.00	1.15 (1.20)
2	Well	0.20	Borehole	0.40	0.30 (0.14)	Well	2.00	Borehole	2.00	2.00 (0.0)
3	Borehole	0.04	Borehole	0.09	0.07 (0.04)	Borehole	0.30	Well	1.00	0.65 (0.49)
4	Borehole	0.15	Well	0.40	0.28 (1.8)	Borehole	0.20	Well	0.20	0.20 (0.0)
5	Borehole	0.09	Borehole	0.07	0.08 (0.01)	Borehole	0.10	Well	0.10	0.10 (0.0)
6	Well	0.30	Borehole	0.50	0.40 (0.14)	Well	0.40	Borehole	0.20	0.30 (0.14)
7	Borehole	0.20	Borehole	0.10	0.15 (0.07)	Well	0.20	Borehole	0.40	0.30 (0.14)
8	Well	0.20	Well	0.10	0.15 (0.07)	Borehole	0.40	Borehole	0.30	0.35 (0.07)
9	Well	0.15	Well	0.20	0.18 (0.04)	Well	0.60	Borehole	0.09	0.34 (0.36)
10	Borehole	0.30	Well	0.20	0.25 (0.07)	Well	0.30	Borehole	0.30	0.30 (0.0)
11	Borehole	0.10	Borehole	0.20	0.15 (0.07)	N/A	N/A	N/A	N/A	N/A
12	Well	0.50	Borehole	1.00	0.75 (0.35)	N/A	N/A	N/A	N/A	N/A

Appendix D: Mean (±SD) fluoride concentration (mg/L) of water samples collected from wells and boreholes in Ibadan North (Urban) and Ibarapa Central (Rural) LGAs (Stage 2 of Pilot Study).

Local Government Areas	Ward No.	Area 1		Area 2		Area 3		Area 4		Area 5		Mean (±SD) (mg/L)
		Water source	F conc (mg/L)	Water source	F conc (mg/L)	Water source	F conc (mg/L)	Water source	F conc (mg/L)	Water source	F conc (mg/L)	
Ibadan North (Highest urban F in Stage 1)	2	Well	0.60	Borehole	0.40	Borehole	0.20	Well	0.20	Well	0.40	0.36 (0.17)
	6	Borehole	0.30	Borehole	0.80	Well	0.30	Borehole	1.0	Well	0.20	0.52 (0.36)
	10	Borehole	0.03	Well	0.09	Well	0.20	Borehole	0.06	Well	0.08	0.09 (0.06)
	12	Borehole	0.04	Well	0.03	Borehole	0.20	Borehole	0.15	Well	0.30	0.14 (0.11)
Ibadan North (Lowest urban F in Stage 1)	3	Borehole	0.10	Well	0.20	Well	0.25	Borehole	0.20	Well	0.20	0.19 (0.05)
	5	Well	0.30	Borehole	0.20	Well	0.19	Borehole	0.20	Borehole	0.10	0.20 (0.07)
	7	Well	0.30	Well	0.20	Well	0.40	Borehole	0.10	Well	0.20	0.24 (0.11)
	8	Well	0.30	Borehole	0.20	Well	0.20	Well	0.10	Borehole	0.20	0.20 (0.07)
Ibarapa Central (Highest rural F in Stage 1)	1	Borehole	2.00	Borehole	0.30	Borehole	3.00	Well	2.0	Well	0.30	1.52 (1.19)
	2	Well	0.90	Well	0.60	Well	0.50	Borehole	0.90	Well	0.60	0.70 (0.19)
	3	Well	0.30	Borehole	0.30	Borehole	0.30	Well	0.40	Well	3.00	0.86 (1.20)
	8	Well	0.35	Borehole	0.25	Well	0.40	Borehole	0.30	Well	0.45	0.35 (0.08)
Ibarapa Central (Lowest rural F in Stage 1)	4	Well	0.09	Well	0.10	Well	0.10	Borehole	0.07	Well	0.10	0.09 (0.01)
	5	Borehole	0.50	Well	0.20	Borehole	0.15	Well	0.06	Borehole	0.20	0.22 (0.17)
	6	Borehole	0.80	Well	0.60	Borehole	0.75	Well	2.00	Well	0.30	0.89 (0.65)
	7	Well	0.60	Borehole	0.07	Well	0.20	Borehole	0.50	Borehole	0.90	0.45 (0.33)

Appendix E – Ethical Approval Newcastle University.

03 August 2012

Olushola Ibiyemi
Research Office 4.026
Level 4
School of Dental Sciences

Faculty of Medical Sciences

Newcastle University
The Medical School
Framlington Place
Newcastle upon Tyne
NE2 4HH United Kingdom

Professor Michael Whitaker
FIBiol FMed Sci
Dean of Research & Innovation

FACULTY OF MEDICAL SCIENCES: ETHICS COMMITTEE

Dear Olushola

Title: Factors associated with the occurrence of Developmental Defects of Enamel and Dental Fluorosis among 4 and 8 year-olds in Nigeria
Application No: 00553/2012
Start date to end date: 01 October 2012 to 14 November 2014

On behalf of the Faculty of Medical Sciences Ethics Committee, I am writing to confirm that the ethical aspects of your proposal have been considered and your study has been given ethical approval.

The approval is limited to this project: **00553/2012**. If you wish for a further approval to extend this project, please submit a re-application to the FMS Ethics Committee and this will be considered.

During the course of your research project you may find it necessary to revise your protocol. Substantial changes in methodology, or changes that impact on the interface between the researcher and the participants must be considered by the FMS Ethics Committee, prior to implementation.*

At the close of your research project, please report any adverse events that have occurred and the actions that were taken to the FMS Ethics Committee.*

Best wishes,

Yours sincerely

M. Holbrough

Marjorie Holbrough
On behalf of Faculty Ethics Committee

cc.
Professor Michael Whitaker, Dean of Research & Innovation
Ms Lois Neal, Assistant Registrar (Research Strategy)

*Please refer to the latest guidance available on the internal Newcastle Biomedicine web-site.

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www.ncl.ac.uk

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Appendix F – Ethical Approval UI/UCH Nigeria.

INSTITUTE FOR ADVANCED MEDICAL RESEARCH AND TRAINING (IAMRAT)
COLLEGE OF MEDICINE, UNIVERSITY OF IBADAN. IBADAN, NIGERIA.
Director: **Prof. A. Ogunniyi,** B.Sc(Hons), MBChB, FMCP, FWACP, FRCP (Edin), FRCP (Lond)
Tel: **08023038583, 08038094173**
E-mail: **aogunniyi@comui.edu.ng**

UI/UCH EC Registration Number: **NHREC/05/01/2008a**

NOTICE OF FULL APPROVAL AFTER FULL COMMITTEE REVIEW

Re: Factors Associated with the Occurrence of Developmental Defects of Enamel and Dental Fluorosis among 4 and 8 year Olds in Nigeria

UI/UCH Ethics Committee assigned number: UI/EC/12/0244

Name of Principal Investigator: **Dr. O. Ibiyemi**

Address of Principal Investigator: Department of Periodontal & Community Dentistry, College of Medicine, University of Ibadan, Ibadan, Nigeria

Date of receipt of valid application: 07/08/2012

Date of meeting when final determination on ethical approval was made: **N/A**

This is to inform you that the research described in the submitted protocol, the consent forms, and other participant information materials have been reviewed and *given full approval by the UI/UCH Ethics Committee.*

This approval dates from 06/11/2012 to 05/11/2013. If there is delay in starting the research, please inform the UI/UCH Ethics Committee so that the dates of approval can be adjusted accordingly. Note that no participant accrual or activity related to this research may be conducted outside of these dates. *All informed consent forms used in this study must carry the* UI/UCH EC *assigned number and duration of* UI/UCH EC *approval of the study.* It is expected that you submit your annual report as well as an annual request for the project renewal to the UI/UCH EC early in order to obtain renewal of your approval to avoid disruption of your research.

The National Code for Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations and with the tenets of the Code including ensuring that all adverse events are reported promptly to the UI/UCH EC. *No changes are permitted in the research without prior approval by the* UI/UCH EC *except in circumstances outlined in the Code. The* UI/UCH EC *reserves the right to conduct compliance visit to your research site without previous notification.*

UI/UCH EC
06 NOV 2012
APPROVED
COLLEGE OF MEDICINE

Professor A. Ogunniyi
Director, IAMRAT
Chairman, UI/UCH Ethics Committee
E-mail: uiuchirc@yahoo.com

Appendix G – Letter of Approval from Ministry of Education, Nigeria.

TELEPHONE: IBADAN

PRIVATE MAIL BAG NO 5014

MINISTRY OF *EDUCATION*

SCHOOLS .....DEPARTMENT

IBADAN, OYO STATE OF NIGERIA

*Your Ref. No*_____
All correspondence should be addressed to the Hon. Commissioner Quoting. **EDU. 215T7/47**
*Our Ref. No*_____

11th January, 2013

Olushola Ibiyemi

Department of Periodontology and Community Dentistry

College of Medicine

University of Ibadan

RE: PERMISSION TO UNDERTAKE A RESEARCH AMONG CHILDREN IN NURSERIES AND PRIMARY SCHOOLS

I am directed to refer to the above subject and convey the Ministry's approval to conduct research on "Factors associated with the occurrence of developmental defects of enamel among 4 and 8 year olds" in nurseries and primary schools respectively. Pupils who meet the requirement in nurseries and primary schools in Ibadan and Ibarapa should be recruited for this study. However, consent of the selected students must be gained from their parents or legal guardians. You are to liaise with the Local Inspector of Education of the Local Governments in Ibadan and Ibarapa before you commence the study.

Yours Sincerely

Alabi

Mr M.A. Alabi

For Honourable Commissioner for Education

Appendix H– Letter to parent/legal guardian (English).

Dear Parent/Guardian,

Field work on "Causes of tooth problems in 4 and 8 year old children in Nigeria".

My name is Olushola Ibiyemi. I am a Nigerian Dentist carrying out a research that will find out if fluoride from foods and drinks and genetics has an effect on fluoride that is pass out in urine and tooth problems among 4 and 8 year olds in Nigeria. This information will help in the use of fluoride in children.

The research will be in two stages: Stage 1 includes firstly, collection of information through a short interview about your socioeconomic status and food and drink consumption and tooth cleaning practices of your child/ward. Secondly, your child's height and weight will be recorded and his/her mouth will be checked at his/her school/nursery to record the presence of tooth problems by a registered Nigerian. Photographs of your child's teeth without including the whole of his/her face so as to hide his/her identity when the picture of the teeth is looked at in more detail will be taken. Your child may be asked to take part in Stage 2 and you will be asked to collect his/her 24 hour urine. In addition, a soft brush will be used to rub on the inner surface of your child's cheek in non-injurious and painless way so as to collect cells that will only be used to show genes (substances in the body) that are linked to a kind of tooth discoloration by the Nigerian registered Dentist. The examination of the mouth and the rubbing of the cheek will be carried out using hygienic materials. Following the study, you will be given health education on how to take care of your child's teeth and mouth and your child will also be given this education. If your child has any problem with his/her teeth and mouth, you will be properly advised on how the problem will be solved. You or your child may withdraw from the study at any time, without any harm. It is important to note that your personal, family and child's identity will not be divulged. Only the research team will have access to the information collected and it will be stored securely. All the information will be kept confidential. This project was approved by the Ministry of Education and the Local Government Education Board. Attached is the participant information sheet for further explanation of the study. I should be grateful if you can attend a meeting at the school/nursery premises where you will be given more details of the study and have the opportunity to ask questions. Then you will be allowed to think about you and your child's participation before written informed consent is obtained from you. If you are willing to participate, you will sign or thumb print the consent form attached to this letter. Your child can also assent to his/her participation if he/she wishes. Please, if you have queries about this project, can you contact me on this telephone number: 08037201253 or through the head teacher of your child's school or nursery. Thank you very much for taking time to read this letter. I hope to meet you and your child soon.

Yours sincerely,

Olushola Ibiyemi (BDS, FMCDS, MPH)
PhD Student, Newcastle University, UK

Appendix I – Participant Information Sheet (English).

Parent or Legal Guardian and 8 year olds

SECTION 1: Project Details

Project Title	Causes of tooth problems among 4 and 8 year olds in Nigeria.	
	Name	Olushola Ibiyemi
	Email Address	o.ibiyemi2@ncl.ac.uk
	Contact Address	Child Dental Health School of Dental Sciences University of Newcastle NE2 4HH
Sponsoring institution	Newcastle University	
If ethical approval has been obtained		
Funding source	Newcastle University	
How to file complaint	If you have any concerns or are uncomfortable about any aspect of this study, please contact the researcher Dr Olushola Ibiyemi through the head teacher of your child's school or on telephone number 08037201253, email **o.ibiyemi2@ncl.ac.uk** in the first instance. You can also contact the researcher at project meetings in your child's school or nursery. You can contact the lead supervisor Dr Anne Maguire email anne.maguire@ncl.ac.uk, if you have email access. Newcastle University has agreed to provide indemnity insurance for this study.	

SECTION 2: Purpose/aims of the research

The aim of the research is to find out if fluoride from foods and drinks and genetics has an effect on fluoride that is pass out in urine and tooth problems among 4 and 8 year olds in Nigeria.

SECTION 3: Possible conflicts of interest

There is no conflict of interest

SECTION 4: Participant involvements

Stage 1 You will be interviewed using a questionnaire about your socioeconomic status and continuous residence of your child in the community where you live now. Information on food and drink consumption and tooth cleaning practices of your child will also be obtained from you. This interview will last for 10 minutes. Secondly, your child's height and weight will be recorded and his/her mouth will be checked at his/her school/nursery to record the presence of tooth problems by a Nigerian registered dentist. Photographs of your child's teeth without including the whole of his/her face so as to hide his/her identity when the picture of the teeth is looked at in more detail will be taken. . These measurements, examination and photograph taking will take about 20 minutes and your child will be given regular breaks during this process.

Stage 2
A 24 hour urine of your child will be collected at home by you and at school by a research assistant. The urine will be checked for fluoride levels. In addition, a soft brush will be used to rub on the inner surface of your child's cheek in non-injurious and painless way to collect cells for genetic analysis by the Nigerian registered Dentist.
Stage 3
The genetic analysis will only be used to identify genes (substances in the body) that are shown in a kind of tooth discoloration. This collection of cells will take 5 minutes.

You will thumb print or sign a written consent form before you and your child will participate. Your child will also assent to his/her participation. This consent and assent will be recorded by verbal witness.

SECTION 5: Exclusion criteria (reasons why a person should/could not take part)

- Parents or guardians of participants who have medical conditions that would affect their ability to give appropriate informed consent will be excluded.
- Children with underlying medical problem, including metabolic and renal diseases.
- Children who have received dietary fluoride supplements or professionally applied fluorides in the past 3 months.
- Children who have not been continuously living in the selected study area since birth.
- Children whose family will plan to move from the area of residency during study.
- Children who are suffering from bed-wetting are excluded.

SECTION 6: Benefits and risks

Benefits: The study will not benefit your child or ward directly but the information I will get will help to provide information on fluoride use in children. You and your child will receive a certificate of participation in this research, toothpaste and toothbrush and an oral health education.
Risks: There are no foreseeable risks from taking part in this research because all the procedures for collecting information will not cause any harm or injury.

SECTION 7: Terms for withdrawal

Your participation and that of your child is entirely voluntary. If you and your child decide to take part, both of you will be given this information sheet to keep and you will be asked to sign or thumb print on a consent form. Your child will be invited to assent to his/her participation. If you or your child is not comfortable during any point in the research session, you may leave without giving a reason. You and your child/ward can withdraw at any time without giving reasons and both of you will not be penalised for withdrawing nor will you be questioned on why you have withdrawn. If either of you choose to leave the research, you can give permission for the researcher to use data collected or you can request it is deleted.

SECTION 8: Usage of the data

Your personal information and those of your child will be kept strictly confidential during the research and dissemination of research findings. Non-identifiable participant data will be stored on University and personal computers. No subject identifiable data will leave the study site. All testing materials will contain the participant's unique study code only. Consent forms that contain identifiable data will be stored in a separated locked filing cabinet to the non-identifiable data. The quality and retention of study data will be the responsibility of the researcher. No other researcher will have access to identifiable data without you and your child's permission. Other researchers will have access to this data only if they agree to preserve the confidentiality of the data and if they agree to the terms specified in this form. Genetic material i.e. cells collected when the soft brush is rubbed in the cheek of your child will not bear the name of your child and will be destroyed after the findings of research have been published.

Appendix J – PIS – Collection of 24 hour urine sample.

The importance of this stage:
Fluoride is ingested from foods, drinks and possibly toothpastes or chewing sticks. Some fluorides are taken into body cells and tissues while some are passed out through urine. To estimate the amount of fluoride taken into your child's body, I will need to know the amount of fluoride that is passed out through urine which will then be subtracted from the total fluoride ingested by your child.

Steps for collecting and storing the 24 hour urine

- A 3 litre-potty, funnel and a 2 litre-collection bottle will be given to you to collect your child's urine. Your child will pass his/her urine into the potty at the toilet and the urine will then be poured into the collection bottle using the funnel. The collection bottle should be tightly closed and the potty and funnel rinsed with water each time the urine is collected. The bottle of the urine should be kept near the toilet if possible and out of the reach of children.
- At home, very early in the morning as child wakes up, you will ensure that the time that your child passes his/her first urine is recorded. This first urine should not be collected. A reminder will be sent to you.
- Thereafter, monitor your child for urine collection before he/she goes to school, preferably encouraging your child to pass urine just before going to school or nursery.
- At school or nursery, the research assistant will encourage your child to pass urine just after your child arrives at school/nursery and thereafter the research assistant will monitor and supervise the collection of urine throughout school hours.
- The research assistants will also encourage your child to pass urine just before he/she leaves school/nursery and are about to go home.
- At home, you should encourage your child to pass urine just when he/she gets home and he/she is closely monitored before going to bed. A reminder will be sent to you.
- You should ensure that urine is collected lastly before going to bed and firstly after child gets out of bed. A reminder will be sent to you.
- The research assistant will visit you at the end of the 24 hour collection period to collect your child's urine sample.
- The research assistant will then measure the quantity of the urine produced and arrange for the fluoride analysis to be done.

If you have any problem during the collection of the urine, you can contact Olushola Ibiyemi on 08037201253.
Thank you.

Appendix K - Consent form (English).

Project title: Causes of tooth problems among 4 and 8 year olds in Nigeria.

I, the undersigned, confirm that (please tick box as appropriate):

1.	The information about this research has been read to me. I have also read and understood the information about the research, as provided in the information sheet dated ……	
2.	I have been able to ask questions about my participation in this research.	
3.	I voluntarily agree to participate and allow my child to participate in the research.	
4.	I understand that I and my child can withdraw at any time without giving reasons and we will not be penalised for withdrawing nor will we be questioned on why we have withdrawn.	
5.	The procedures regarding confidentiality have been clearly explained (e.g. use of names, pseudonyms, anonymisation of data) to me.	
6.	The use of the data in research, publications, sharing and archiving has been explained to me.	
7.	I understand that other researchers will have access to this data only if they agree to preserve the confidentiality of the data and if they agree to the terms I have specified in this form.	
8.	I, along with the researcher agree to sign and date this informed consent form.	

Participant:

…… …… ……..

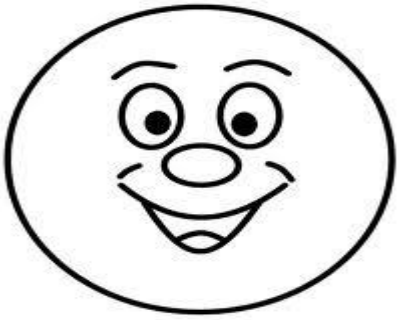
Name of participant Signature Date

Researcher:

…… …… ……..

Name of researcher Signature Date

Assent for children (Children can tick any part of the diagram or draw their own picture if they would like to take part in the research).

	
You can draw your own picture here	**Or you can tick (√) this picture if you would like to take part.**

PLEASE KEEP A COPY OF THE SIGNED INFORMED CONSENT.

Appendix L – Gantt Chart of PhD Field work.

Month	**January**					**February**				**March**				**April**				**May**					**June**			
Week	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**
Location				**Area 1**						**Area 2**				**Area 3**				**Area 4**								
Setting up	x																									
Purchase/ordering of materials	x																									
Printing and distribution of letters to Institutional Authorities		x																								
Recruitment and training of research team		x																								
Distribution of delegation log		x																								
Distribution of invitation letters, PIS and consent forms			x							x				x				x								
Meeting with parents			x							x				x				x								
Distribution of 2 universal bottles for collection of drinking and cooking water			x							x				x				x								
Provision and recording of participants identification numbers			x							x				x				x								
Phase 1																										
Height and weight measurements				x	x						x	x			x	x				x	x					
DMFT/dmft				x	x						x	x			x	x				x	x					
DDE (Extent)				x	x						x	x			x	x				x	x					
TFI				x	x						x	x			x	x				x	x					
Deans fluorosis score				x	x						x	x			x	x				x	x					
Digital photography				x	x						x	x			x	x				x	x					
Interviewing parents – Dental Health & Nutrition				x	x						x	x			x	x				x	x					
Collection of drinking and cooking water from parents				x	x						x	x			x	x				x	x					
Phase 2																										
Check record book to see if child was selected for phase 2						x							x				x					x				
Interviewing parents – Food Frequency Questionnaire						x							x				x					x				
Distribution of food collection materials						x							x				x					x				
Distribution of funnels						x							x				x					x				
Phase 3																										
Buccal swab						x							x				x					x				
Storage of buccal swabs in -80ºC						x							x				x					x				
Reminder to parents to collect urine at home							x							x				x					x			
Urine collection at school							x							x				x					x			
Urine collection at home and delivery of urine in laboratory							x							x				x					x			
Fluoride analysis of water and urine							x	x	x					x	x	x		x	x	x			x	x	x	

Appendix M - Letter of Authorisation DEFFRA.

Authorisation No: TARP/2012/451

DEPARTMENT FOR ENVIRONMENT, FOOD AND RURAL AFFAIRS

AUHORISATION FOR THE IMPORTATION FROM THIRD COUNTRIES OF RESEARCH SAMPLES

European Communities Act 1972

TRADE IN ANIMALS AND RELATED PRODUCTS REGULATIONS 2011

The Secretary of State for Environment, Food and Rural Affairs, by this authorisation issued under the terms of Paragraph 4 of Schedule 3 of the Trade in Animal and Related Products Regulations 2011 authorises:

Research Office 4.026 Level 4 Dental School Framlington Place Newcastle University NE2 4BW	Name and full postal address

subject to and in accordance with the conditions set out below, the landing in England of

Processed Meat Based Products Containing Bovine & Domestic Fowl, intended for particular studies or analyses	Product

from

Nigeria	Countries of origin

at

Newcastle Airport	Ports of entry

until

19th December 2013	Expiry Date

Dated: 19th December 2012

Officer of the Department for Environment, Food and Rural Affairs

Appendix N – Anthropometric measurement and Dental Health Assessment form.

Area Code:

Child identification number:

Height: …… (cm)
Weight: …… (Kg)

Tooth type (Primary)		55	54	53	52	51	61	62	63	64	65	
dmft												
DDE												
TFI												
Tooth type (Permanent)	16	15	14	13	12	11	21	22	23	24	25	26
DMFT												
DDE												
TFI												
TFI												
DDE												
DMFT												
Tooth type (Permanent)	46	45	44	43	42	41	31	32	33	34	35	36
TFI												
DDE												
dmft												
Tooth type (Primary)		85	84	83	82	81	71	72	73	74	75	

Dean's Score for Dental Fluorosis:
(considering the Labial/buccal surfaces of ALL erupted teeth)
dmft/DMFT: ALL ERUPTED TEETH
***DDE: Labial/Buccal surfaces of 10 index teeth**

Appendix O - Questionnaire on socio-demographic data (English).

Area Code:

Child's identification number: | | | | | | |

Child's date of birth: | |

Child's Gender: 1) Male 2) Female | | | | | | |

Date questionnaire was completed

Socioeconomic status of parents or guardians

1. What is your relationship to this child?
    1) Mother
    2) Father
    3) Grandmother
    4) Grandfather
    5) Others (Please specify) ……
2. What is your level of education?
    1) Never went to school
    2) Primary school
    3) Koranic school
    4) Secondary school
    5) Vocational school
    6) Polytechnic/University
3. What is your occupation? ……
4. How much is your average monthly income?
    1) N5,000 - N10,000
    2) N11,000 - N20,000
    3) N21,000 - N30,000
    4) N31,000 - N40,000
    5) Greater than N40,000
5. How long has your child/ward been living in this locality?
    1) Since birth
    2) Less than 4 years
    3) 4 years
    4) 5 years
    5) 6 years
    6) 7 years
    7) 8 years

6. Did your child have a normal birth

   (1). Yes (2). No

7. If no, what kind of problems did the mother/baby have at birth

   (1). Premature

   (2). Low birth weight

   (3). Maternal medical problem

   (4). Others (Please specify)……

8. What was your child's birth weight: ……Kg
9. Where was your child born
   (1). Hospital

   (2) At home

   (3) Traditional home

   (4) Others (Please specify) ……

10. If child was born in the hospital, what type of hospital was the child born: …….and on what day: ……
11. Was your child (1) Exclusively breast fed (2) Exclusively formula-fed (3) Mixed feeding
12. If your child was exclusively breast-fed, at what age did your child stop exclusive breast-feeding ……
13. If your child was exclusively breast fed and later stopped breast feeding, what kind of foods and drinks did you introduce ……
14. If your child was exclusively breast fed and at what age did you stop breast feeding him/her completely ……
15. If your child was exclusively formula fed, at what age did you stop exclusive formula feeding ……
16. If your child was exclusively formula fed and later stopped formula feeding, which types of foods and drinks did you introduce ……
17. If your child was exclusively formula fed and at what age did you stop formula feeding him/her completely ……
18. If your child took formula milk, which type was it (1) Powdered 2) Liquid
19. If powdered milk, which type of water did you use to mix it (1) Tap water (2) Well/borehole water (3) Bottled/sachet water
20. Did you bottle feed your child with sugary drinks (1) Yes (2) No
21. If you bottle feed your child with sugary drinks, list the types of sugary drinks ……
22. If you bottle feed your child with sugary drinks, what time of the day did you do so.
    (1) Day time
    (2) Night time
    (3) Both
23. If you bottle feed your child with sugary drinks, what age did you stop doing it? …
24. List the kind of weaning foods that your child was given? ……
25. List the kind of weaning drinks that your child was given? …
26. List the commonly consumed foods your child currently eats? ……
27. List the commonly consumed drinks your currently child drinks? ……
28. Did your child attend child care at any stage? (1) Yes (2) No
29. If yes to question 22, at what age did your child attend child care? ……
30. If yes to question 22, how many days per week did your child attended child care? …

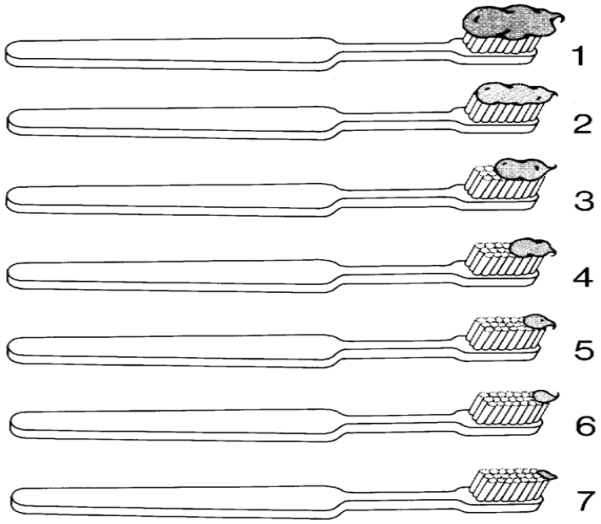
31. If yes to question 22, was your child fed at child care (1) Yes (2) No
32. If yes to question 22, did you send any food/drink to child care for your child (1)Yes (2) No
33. Has your child ever had any of the following diseases or conditions?

Diseases/conditions	Yes	If yes, at what age	No	Don't know
Whooping cough				
Neonatal tetanus				
Pneumonia				
Rheumatic fever				
Chicken pox				
Diarrhoea				
Measles				
Accident/trauma (face)				
Accident/trauma (Teeth)				
Mental disorder				
Bronchial asthma				
Recurrent viral infection				
Kidney disease				
Others (Please specify) ……				

34. At what age did your child/ward start to clean his/her teeth……
35. With what did your child use to clean his/her teeth mostly
(1) Toothpaste
(2) Chewing sticks
(3) Toothpaste and chewing sticks

If your child uses toothpaste, please answer questions 36 – 40

36. If your child/ward uses toothpastes, what brand of toothpaste is it?
(1) Macclean
(2) Close up
(3) Darbul
(4) Colgate
(5) Crest
(6) Others (Please specify).....
37. Parent/legal guardian to show toothpaste label to interviewer who should record fluoride concentration on the label here: .....
38. If your child/ward uses toothpaste, select the amount used per brushing from figure below and record in the box below:



39. If you child/ward uses toothpaste, how many times does he/she uses it daily?
    (1) Once (2) Twice (3) Three or more times
40. If your child/ward uses toothpaste the most, does he/she
    (1) Rinse after use
    (2) Spit after use
    (3) Does not spit or rinse
41. If your child/ward uses chewing stick, how many times per day does he/she uses it?
    (1) Once (2) Twice (3) Three times (4) Four times (5) Others (Specify) ……
42. If your child/ward uses chewing stick, name of chewing stick……..
43. Does any member in your child/ward family has discoloured teeth?
    (1) Yes (2) No
44. If yes to question 44, which member of the family?

Members of child's family	Yes	No
Members of paternal family		
Members of maternal family		
Your child's sibling		
Others (Please specify).....		

Questionnaire on socio-demographic data (Yoruba).

Area Code: | | | |

Child's identification number: | | | |

Kini ojo ibi omo yi: | | | | | | |

Okunrin tabi obirin: 1) Okunrin 2) Obirin | |

Date questionnaire was completed | | | | | | |

Socioeconomic status of parents/legal guardians

1. Bawo le se je si omo yi?
   (1) Mama re
   (2) Baba re
   (3) Iya mama re
   (4) Iya baba re
   (5) Elomi (Ejoo esalaye) ……
2. Iwe melo le ka?
   (1) Mi o losi ile iwe rara
   (2) Ile iwe alakobere
   (3) Ile kewu
   (4) Ile iwe girama
   (5) Ile iwe e kose
   (6) Ile iwe Unifasiti
3. Ise wo ni e se?.....
4. E lo ni e ngba losu?
   (1) Egberun marun si egberun mewa losu
   (2) Egberun mokanla si egberun ogun losu
   (3) Egberun mokanlelogun si egberun ogbon losu
   (4) Egberun mokanlelogbon si egberun ogogi losu
   (5) Oju egberun ogogi losu
5. Lati igbawo ni omo yin ti gbe ibi yi?
   (1) Lati igba ti abii

(2) Odun merin seyin
(3) Odun marun seyin
(4) Odun mefa seyin
(5) Odun meje seyin
(6) Odun mejo seyin

6. Nje ebi omo yin lai si isoro Kankan fun yin tabi omo yin
   (1) Beeni (2) Beeko
7. To baje beeko, iru isoro wo ni eeyin tabi omo yin ni
   (1) Ojo oyun re o pe
   (2) Omo o tobi to
   (3) ailera mama
   (4) Imiran (Ejoo esalaye) ....…….....
8. Bawo ni omo yin se tobi to: …….kg
9. Ibo ni eti bi omo yin
   (1) Ile iwosan
   (2) Ni ile
   (3) Ile agbebi ibile
   (4) Imiran (Ejoo esalaye).....
10. Ti o ba je pee bi omo yin ni ile iwosan, iru ile iwosan woo.....ati ojo wo ni ……
11. Se omo yin 1) Mu oyon ni kan b) Mu miliki ni kan c) Mu oyon ati miliki ati ounje
12. Ti o ba je pe oyon ni kan ni omo yin mu, ojo ori woo ni a jawo oyon mumu ni kan …….....
13. Ti o ba je pe oyon ni kan ni omo yim mu, tie de gba lowo re, iru ounje ati nkan mumu wo ni e fun……
14. Ti o ba je pe oyon ni kan ni omo yin mu, omo odun wo ni e gba oyon lenu re patapata …….
15. Ti ob je pe miliki omode ni kan ni omo yin mu, omo odun wo ni e dawo funfun ni miliki omode yi …….
16. Ti ob je pe miliki omode ni kan ni omo yin mu, ti e de dawo funfun, iru ounje ati nkan mumu wo ni e fun ……
17. Ti ob je pe miliki omode ni kan ni omo yin mu, omo odun wo ni e dawo funfun ni miliki omode yi patapata ……
18. Ti omo yin ba mu miliki omode, iru iwo ni 1) Gberefu 2) Olomi
19. Ti o ba je gberefu, iru omi woo ni e fi poo 1) Omi ero 2) Omi kanga tabi boreholu 3) omi inu igo tabi ora
20. Nje e fun omo yin ni nkan didun mumu ni nu feeder (1) Beeni (2) Beeko
21. Ti o ba je pe e fun omo yin ni nkan mumu ti ti o dun ni feeder, e so awon nkan didun yi …….
22. Ti o ba je pe e fun omo yin ni nkan mumu ti o dun ni feeder, akoko igba wo ni e ma fun
   (1) Ni oju mo
   (2) Ni ale
   (3) Ni oju mo ati ni ale
23. Ti o ba je pe e fun omo yin ni nkan mumu ti o dun ni feeder, omo odun wo ni o to ti e fi fun mu mo ……
24. E so awon ounje ti omo yin je ni igba ti e fe gba omu ni enu re? ……

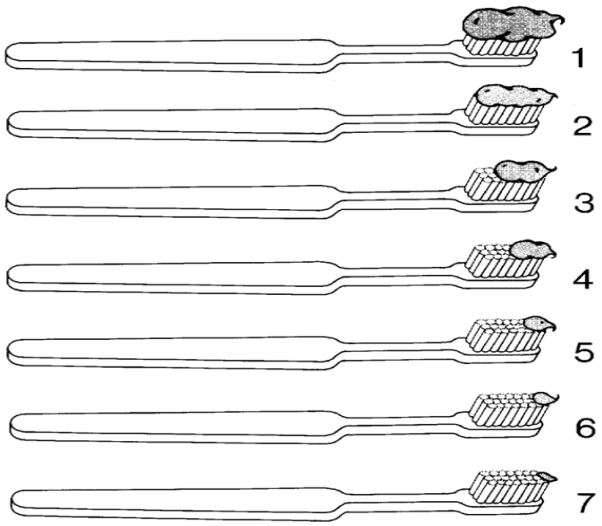
25. E so awon nkan ti e fun omo yin mu ni igba ti e fe gba omo ni enu re?…
26. E so awon ounje ti omo yin ma saba je ju ni sisiyi? ……
27. E so awon nkan ti omo yin ma saba mu ju ni sisiyi? .....
28. Se emu omo yin losi day care ni igba ti e nto? 1) Beeni 2) Beeko
29. Ti o ba je pe emu omo yin losi day care ni igba ti e nto, omo oodun wo ni e mu lo ni day care? .....
30. Ti o ba je pe emu omo yin losi day care ni igba ti e nto, o jo melo lose ni e ma mu losi ibe?..
31. Ti o ba je pe e mu omo yin losi day care ni igba ti e nto, se won ma nfun ni ounje ni ibe 1) Beeni 2) Beeko
32. Ti o ba je pe e mu omo yin losi day care ni igba ti nto, se e ma fi ounje ranse losi day care fun 1) Beeni 2) Beeko
33. Se omo yin ti se i ru awon aisan yii?

Aisan	Beeni	Ti o ba je beeni, ni ojo ori wo lose	Beeko	Emi o mo
Iko awu bi				
Arun ipaa				
Otutu aya				
Iba arumoleegu				
Tita				
Igbe gburu				
Eyii				
Ijamba (Oju)				
Ijamba (Eyin)				
Arun opolo				
Iko semisemi				
Arun bu shegede				
Aisan kidirin				
Imiran (E salaye) ……				

34. Ojo ori wo ni omo yin bere si foo eyin re? ……
35. Nkan wo ni omo yin fin maa foo eyin re ju?
    (1) Burosi ati ose ifo eyin
    (2) Paako
    (3) Ose ifo eyin ati paako

Ti o ba je pe omo yin ma lo burosi ati ose ifo eyin, e daun ibere 36 - 40

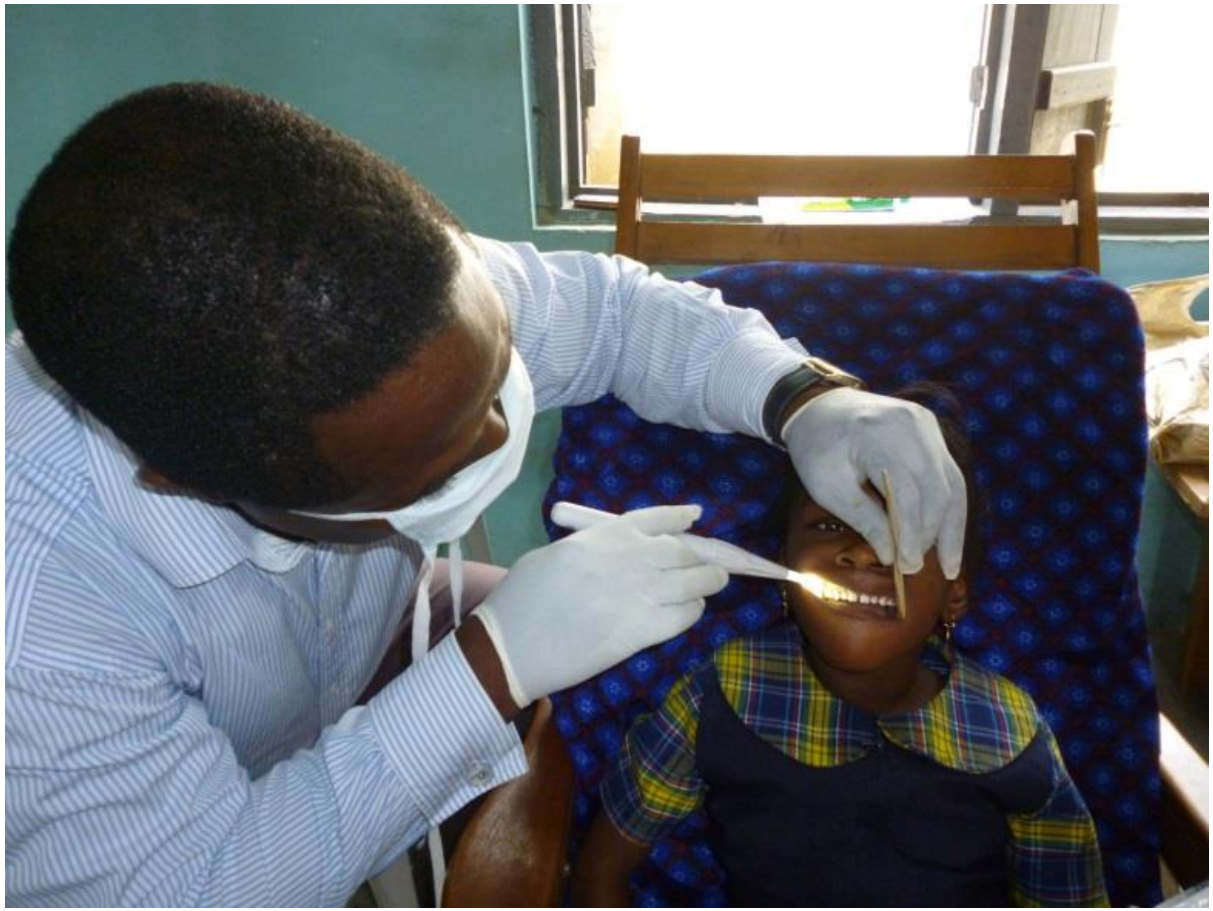
36. Ti o ba je pe omo yin ma nlo burosi ati ose ifo eyin, i ru wo ni?
    (1) Maccleans (2) Close up (3) Darbul (4) Colgate (5) Crest (6) Others (Specify) ……
37. Ki obi tabi alagbato f ii ru ose ifo eyin ti omo ma nlo han olubere ki o ba le ko iru nkan ti won fi se ose if o eyin yi .....
38. Ti omo yin ban lo ose if o eyin, e mu iye ti o ma nlo lati awon aworan yi: ☐



39. Ti omo yin ban lo ose i fo eyin, e me lo loju mo kan ni o ma nlo?
    (1) Ee kan (2) Ee meji (Ee meta
40. Ti omo yin ban lo ose ifo eyin se o ma
    (1) San enu re to ba fo tan
    (2) Tu ose if foe yin jade to ba fo tan
    (3) Kii tu tabi san jade
41. Ti omo yin ban nlo paako, bawo ni e yi ti ma nlo se gun to: ……
42. Ti omo yin ban lo paako, kini oruko paake naa?
43. Nje e ni Kankan ni inu ebi omo yin ni eyin ti awo re o dara to?
    (1) Beeni (2) Beeko
44. Ti o ba je beeni si ibere 43, ebi wo ni?

Awon ebi omo	Beeni	Beeko
Ebi baba omo yin		
Ebi iya omo yin		
Egbon tabi aburo omo yin		
Imiran (E salaye)		

Appendix P – Clinical Examination.



Appendix Q - Indices for measurement.

DMFT/dmft index		Developmental defects of enamel		Dean's index		Thylstrup-Fejerskov index (TFI)	
Criteria	**S**	**Criteria**	**S**	**Criteria**	**S**	**Criteria**	**S**
Decayed-permanent tooth	D	Normal	0	Normal: Enamel represents usual translucent semi-vitriform structure type. Surface smooth, glossy and usually a pale creamy white colour.	0	Normal translucency of enamel remains after prolonged air-drying	0
Missing due to caries – permanent tooth	M	Demarcated opacity: Normal enamel thickness, intact surface, varying degree of alteration in translucency of enamel which can be white, cream, yellow, or brown. Alteration is demarcated from adjacent normal enamel with distinct and clear boundary	1	Questionable: Enamel discloses slight aberrations from translucency of normal enamel ranging from a few white flecks to occasional white spots	1	Narrow white lines corresponding to the perikymata	1
Filled due to caries – permanent teeth	F	Diffuse opacity: varying degree of alteration in translucency of enamel usually white with no clear boundary between the adjacent normal enamel. Opacity can be linear or patchy or confluent	2	Very mild: small opaque, paper white areas scattered irregularly over the tooth but not involving as much as approximately 25% of tooth surface. Also teeth showing no more than about 1-2 dm of white opacity at tip of summit of cusps of bicuspid or second molars	2	Smooth surfaces: More pronounced lines of opacity that follow the perikymata. Occasionally confluence of adjacent lines	2
Decayed-primary tooth	d	Hypoplasia: Defect on enamel surface in form of (a) pits- single or multiple, shallow or deep, scattered or in rows or horizontal (b) grooves – single or multiple, narrow or wide (c) partial or complete 4absence of enamel. Affected enamel may be translucent or opaque	3	Mild: White opaque areas in enamel of teeth are more extensive but do not involve as much as 50% of tooth	3	Smooth surfaces: Merging and irregular cloudy areas of opacity. Accentuated drawing of perikymata often visible between opacities	3
Missing due to caries – primary tooth	m	Other defects eg white spot decay	4	Moderate: all enamel surfaces of teeth are affected and surfaces subject to attrition show marked wear. Brown staining frequently a disfiguring feature.	4	Smooth surfaces: the entire surface exhibits marked opacity or appears chalky white. Parts of surface exposed to attrition appear less affected.	4
Filled due to caries – primary teeth	f	Demarcated and diffuse opacities	5	Severe: All enamel surfaces are affected and hypoplasia is so marked that general form of tooth may be altered. Major diagnostic sign of this classification is presenting a corroded appearance	5	Smooth surfaces: Entire surface displays marked opacity with focal loss of outermost enamel (pits) <2mm in diameter	5
Excluded/assessment cannot be made	9	Demarcated opacity and hypoplasia	6	Excluded	8	Smooth surfaces: Pits are regularly arranged horizontal bands <2mm in vertical extension.	6
Sound tooth	S	Diffuse opacity and hypoplasia	7	Not recorded	9	Smooth surfaces: Loss of outermost enamel in irregular areas involving <1/2 of entire surface.	7
Fractured (Trauma)	T	All 3 conditions	8			Smooth and occlusal surfaces: Loss of outermost enamel involving >1/2 of surface	8
		Not recorded	9			Smooth surfaces: Loss of main part of enamel with change in anatomic appearance of surface cervical rim of almost unaffected enamel is often noted.	9
						Smooth surfaces: Loss of outermost enamel in irregular areas involving <1/2 of entire surface.	

Appendix R - Coding sheet.

Dental Health Questionnaire key and coding Sheet

No.	Opt.	Code	No.	Opt.	Code	No.	Opt.	Code
19m	1)Yes, 2)No **Yam/maize – R**	2	22b	1)Yes, 2)No **Tea**	2	28gii	Mths or N/A	48
19n	1)Yes, 2)No **Confectioneries**	2	22c	1)Yes, 2)No **Herbal tea**	2	28hi	1)Yes, 2)No, 3)Don't know **Trauma – face**	2
20a	1)Yes, 2)No **Water**	1	22d	1)Yes, 2)No **Coffee/chocolat**	2	28hii	Mths or N/A	N/A
20b	1)Yes, 2)No **Tea**	2	22e	1)Yes, 2)No **Milk/yoghurt – L**	2	28ii	1)Yes, 2)No, 3)Don't know **Trauma – teeth**	2
20c	1)Yes, 2)No **Herbal Tea**	2	22f	1)Yes, 2)No **Milk – P**	2	28iii	Mths or N/A	N/A
20d	1)Yes, 2)No **Coffee/Chocolate**	2	22g	1)Yes, 2)No **Sugared RTD**	1	28ji	1)Yes, 2)No, 3)Don't know **Mental disorder**	2
20e	1)Yes, 2)No **Milk/yoghurt -L**	1	22h	1)Yes, 2)No **Sugarless RTD**	2	28jii	Mths or N/A	N/A
20f	1)Yes, 2)No **Milk – P**	2	22i	1)Yes, 2)No **Liquid conc-Home**	2	28ki	1)Yes, 2)No, 3)Don't know **B. Asthma**	2
20g	1)Yes, 2)No **Sugared RTD**	2	22j	1)Yes, 2)No **Liquid conc-Purchas**	2	28kii	Mths or N/A	N/A
20h	1)Yes, 2)No **Sugarless RTD**	2	22k	1)Yes, 2)No **Powdered conc.**	2	28li	1)Yes, 2)No, 3)Don't know **Recurr. Viral inf.**	2
20i	1)Yes, 2)No **Liquid conc - Home**	2	22l	1)Yes, 2)No **Carbonated drinks**	1	28lii	Mths or N/A	N/A
20j	1)Yes, 2)No **Liquid Conc-Purchas**	2	23	1)Yes 2)No	1	28mi	1)Yes, 2)No, 3)Don't know **Kidney disease**	2
20k	1)Yes, 2)No **Powdered conc**	2	24	1)1-6mths, 2) 7-12mths, 3)>1yr 4)N/A	3	28mii	Mths or N/A	N/A
20l	1)Yes, 2)No **Carbonat. Drinks**	2	25	1)1-4 days, 2) 5-7 days 3)N/A	2	29	1)≤6mths, 2)7-11mths, 3) 12-18mths, 4)>18mths	3
21a	1)Yes, 2)No **Vegetable – C**	1	26	1)Yes 2)No 3)N/A	1	30	1)Toothpaste, 2)Chewing sticks, 3)Both 1 & 2 4) Others	1
21b	1)Yes, 2)No **Fruits**	2	27	1)Yes 2)No 3)N/A	1	31	1))Mac, 2)Cup, 3) Darb, 4)Colg, 5)Cr, 6)OB 7) Max 8)Oth 9) N/A	8
21c	1)Yes, 2)No **Soup**	1	28ai	1)Yes, 2)No, 3)Don't know **W. Cough**	1	32	ppm or N/A	1450
21d	1)Yes, 2)No **Bread**	2	28aii	Mths or N/A	3	33	1-7 or N/A	6
21e	1)Yes, 2)No **Pasta**	1	28bi	1)Yes, 2)No, 3)Don't know **N. tetanus**	2			
21f	1)Yes, 2)No **Rice/beans**	1	28bii	Mths or N/A	N/A	34	1) Once, 2) Twice, 3) >2 times 4)N/A	1
21g	1)Yes, 2)No **Fish/seafoods – C**	2	28ci	1)Yes, 2)No, 3)Don't know **Pneumonia**	2	35	1) rinse, 2) spit, 3) Don't rinse or spit 4)N/A	1
21h	1)Yes, 2)No **Fish/seafoods – R**	2	28cii	Mths or N/A	N/A	36	1)once, 2) twice, 3) thrice, 4) 4times, 5) >4times 6)N/A	6
21i	1)Yes, 2)No **Cereals**	2	28di	1)Yes, 2)No, 3)Don't know **R. fever**	1	37		
21j	1)Yes, 2)No **Meat products - C**	2	28dii	Mths or N/A	36	38		
21k	1)Yes, 2)No **Meat products – R**	2	28ei	1)Yes, 2)No, 3)Don't know **Chicken pox**	2	39	1)Yes, 2)No	1
21l	1)Yes, 2)No **Yam/maize – C**	1	28eii	Mths or N/A	N/A	40a	1)Yes 2)No 3)N/A **Paternal**	2
21m	1)Yes, 2)No **Yam/maize – R**	2	28fi	1)Yes, 2)No, 3)Don't know **Diarrhoea**	2	40b	1)Yes 2)No 3)N/A **Maternal**	1
21n	1)Yes, 2)No **Confectioneries**	2	28fii	Mths or N/A	N/A	40c	1)Yes 2)No 3)N/A **Other siblings**	2
22a	1)Yes, 2)No **Water**	1	28gi	1)Yes, 2)No, 3)Don't know **Measles**	1			

Appendix S - Food Frequency Questionnaire (English).

Area code: | | | |

Child's identification number: | | | |

Group code	Foods/Drinks	Yes (1)/ No(2)	Number of servings per day/week/month/year?	Amount per serving
DG1	Tap water			
DG2	Well/Borehole water			
DG3	Bottled/sachet water			
DG4	Tea (Black tea)			
DG5	Herbal tea eg Lemon grass tea			
DG6	Other hot drinks (Chocolate)			
DG7	Liquid milk/Yoghurt/any other diary liquid drink			
DG8	Powdered milk or any other diary powdered drink			
DG9	Ready to drink (Consumed as purchased - sugared)			
DG10	Ready to drink (Consumed as purchased - sugarfree)			
DG11	Drinks from liquid fruit concentrate you prepared/diluted at home - sugared)			
DG12	Drinks from liquid fruit concentrate you prepared/diluted at home - sugarfree)			
DG13	Drinks from powdered fruit concentrate you prepared/diluted at home - sugared)			
DG14	Carbonated drinks (coke, Fanta, sprite etc)			
FG1	Cooked vegetables			
FG2	Vegetables steamed			
FG3	Fruits			
FG4	Fruits steamed			
FG5	Soup			
FG6	Bread			
FG7	Cooked pasta/spaghetti/noodles			
FG8	Cooked rice & beans dishes			
FG9	Steamed rice & beans dishes			
FG10	Cooked fish or sea foods			
FG11	Fried/roasted fish or sea foods			
FG12	Breakfast cereals			
FG13	Cooked meat or meat products			
FG14	Fried/roasted meat or meat products			
FG15	Cooked yam/cassava/maize			
FG16	Fried/roasted yam/cassava/maize			
FG17	Confectioneries			

Food Frequency Questionnaire (Yoruba).

Area code:

Child's identification number:

Group code	Ounje/Nkan mumu	Beeni (1)/ Beeko(2)	Melo ni ojo/ose/osu/odun	Iye ti omo yin ma nje tabi mu
D1	Omi ero			
D2	Omi kanga/borehole			
D3	Omi inu igo/ora			
D4	Tea alagbada			
D5	Tea ewe lemonu			
D6	Awon nkan mumu ti ogbona (Coffee, chocolate)			
D7	Miliki olomi tabi yoghurt tabi nkan mumu miran lati malu			
DG8	Miliki gberefu tabi miliki gberefu miran lati malu			
DG9	Nkan mum ti oni sugar ti won ti se ti a man ra			
DG10	Nkan mum ti ko ni sugar ti won ti se ti a man ra			
DG11	Nkan mumu ti o lomi to ni sugar ti a se tabi ti a po ni ile			
DG12	Nkan mumu ti olomi ti ooni sugar ti a se tabi ti a po ni ile			
DG13	Nkan mumu lati mkan gberefu ti oni sugar ti a se tabi ti a po ni ile			
DG14	Oti elerindodo (coke, Fanta, sprite etc)			
FG1	Ewebe tabi efo tutu ti ase			
FG2	Ewebe ti abo			
FG3	Eso			
FG4	Eso ti abo			
FG5	Obee alata			
FG6	Buredi			
FG7	Macaroni/spaghetti/noodles ti ase			
FG8	Iresi ati eewa ti ase			
FG9	Iresi ati ewa ti abo			
FG10	Eja tabi ounje oddo ti ase			
FG11	Eja tabi ounje odoo ti a din/yan			
FG12	Ounje oyinbo ti afi nkan agbado se ti omo je laro			
FG13	Eran tabi ounje eran ti ase			
FG14	Eran tabi ounje eran ti a din/yan			
FG15	Isu/ ege/agbado/dodo ti ase			
FG16	Isu/ ege/agbado/dodo ti a din/yan			
FG17	Ikan ninu caki, sweeti, sugar, bisciki, chocolati (Tabataba)			

Appendix T - Data collection sheet (Urine).

Area Code: | | | |

Child's identification number: | | | |

Child's date of birth: | | | |

Child's Gender: 1) Male 2) Female | |

Date questionnaire was completed: | | | |

<u>24 hour urine</u>

Volume of urine collected: ……

Time of first voiding of urine on day 1: …….

Time of last voiding of urine on day 2: ……..

Appendix U: Mean (±SD) age and gender of 302 4-year-old study participants by area.

Age & Gender	Area 1 (Urban, Higher) (n=78)	Area 2 (Rural, Higher) (n=78)	Area 3 (Urban, Lower) (n=76)	Area 4 (Rural, Lower) (n=70)	All Areas (n=302)	p value*	Tukey Post-hoc
Age (years)							
Mean (SD)	4.55 (0.25)	4.48 (0.19)	4.52 (0.24)	4.43 (0.22)	4.50 (0.23)	0.01*	1v4=0.01
Minimum	4.04	4.07	4.08	4.04	4.04		
Maximum	4.97	4.93	4.91	4.90	4.97		
Gender							
Male No. (%)	34 (43.6)	40 (51.3)	46 (60.5)	35 (50.0)	155 (51.3)	0.21+	
Female No. (%)	44 (56.4)	38 (48.7)	30 (39.5)	35 (50.0)	147 (49.7)		
Total No. (%)	78 (100.0)	78 (100.0)	76 (100.0)	70 (100.0)	302 (100.0)		

Notes: * - *One way ANOVA;* + - *Chi-square*

Appendix V: Duration of residence of 4 year-olds in present location by area and gender (n=302).

Duration of residence in Area	Area 1 (Urban, Higher F) (n=78) No. (%)	Area 2 (Rural, Higher F) (n=78) No. (%)	Area 3 (Urban, Lower F) (n=76) No. (%)	Area 4 (Rural, Lower F) (n=70) No. (%)	All areas (n=302) No. (%)	p value*
Male						
From birth	24 (30.8)	40 (51.3)	27 (35.5)	25 (35.7)	116 (38.4)	<0.001
After birth	10 (12.8)	0 (0)	19 (25.0)	10 (14.3)	39 (12.9)	
Total	**34 (43.6)**	**40 (51.3)**	**46 (60.5)**	**35 (50.0)**	**155 (51.3)**	
Female						
From birth	18 (23.1)	35 (44.9)	18 (23.7)	25 (35.7)	112 (31.8)	<0.001
After birth	26 (33.3)	3 (3.8)	12 (15.8)	10 (14.3)	35 (16.9)	
Total	**44 (56.4)**	**38 (48.7)**	**30 (39.5)**	**35 (50.0)**	**147 (48.7)**	
All						
From birth	42 (53.9)	75 (96.1)	45 (59.2)	50 (71.4)	212 (70.2)	<0.001
After birth	36 (46.1)	3 (3.9)	31 (40.8)	20 (28.6)	90 (29.8)	
Total	**78 (100.0)**	**78 (100.0)**	**76 (100.0)**	**70 (100.0)**	**302 (100.0)**	

*Notes: Between males and females (p^*=0.08); * - Chi-square*

Appendix W: Social characteristics (Level of education, occupation and income) of parents or legal guardians of 4 year old study participants by area (n=302).

Social characteristics	Area 1 (Urban, Higher F)(n=78)		Area 2 (Rural, Higher F) (n=78)		Area 3 (Urban, Lower F) (n=76)		Area 4 (Rural, Lower F) (n=70)		Total (n=302)	
	No.	**(%)**	**No.**	**(%)**	**No.**	**(%)**	**No.**	**(%)**	**No.**	**(%)**
Level of education										
Never went to school	25	(32.1)	13	(16.7)	14	(18.4)	19	(27.1)	71	(23.5)
Primary school	19	(24.4)	37	(47.4)	32	(42.1)	27	(38.6)	115	(38.1)
Koranic school	7	(9.0)	0	(0.0)	1	(1.3)	0	(0.0)	8	(2.6)
Secondary school	16	(20.5)	15	(19.2)	25	(32.9)	21	(30.0)	77	(25.5)
Vocational school	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
Polytechnic/University	11	(14.1)	13	(16.7)	4	(5.3)	3	(4.3)	31	(10.3)
Total	**78**	**(100.0)**	**78**	**(100.0)**	**76**	**(100.0)**	**70**	**(100.0)**	**302**	**(100.0)**
Occupation										
Business woman/man	7	(9.0)	0	(0)	2	(2.6)	4	(5.7)	13	(4.3)
Unemployed/housewife	11	(14.1)	2	(2.6)	1	(1.3)	2	(2.9)	16	(5.3)
Trader	37	(47.4)	41	(52.9)	56	(73.7)	45	(64.3)	179	(59.3)
Artisans/farmer	15	(19.2)	24	(30.8)	10	(13.2)	16	(22.9)	65	(21.5)
Civil servant	5	(6.4)	11	(14.1)	4	(5.3)	3	(4.3)	23	(7.6)
Bankers/engineer	1	(1.3)	0	(0.0)	2	(2.6)	0	(0.0)	3	(1.0)
Domestic staff	2	(2.6)	0	(0.0)	1	(1.3)	0	(0.0)	3	(1.0)
Total	**78**	**(100.0)**	**78**	**(100.0)**	**76**	**(100.0)**	**70**	**(100.0)**	**302**	**(100.0)**
Monthly Income										
N5,000 - N10000	44	(56.4)	46	(59.0)	60	(78.9)	50	(71.4)	200	(66.2)
N11000 - N20000	11	(14.1)	16	(20.5)	9	(11.8)	10	(19.0)	46	(15.2)
N21000 – N30000	7	(9.0)	4	(5.1)	2	(2.6)	7	(10.0)	20	(6.6)
N31000 – N40000	2	(2.6)	4	(5.1)	0	(0.0)	0	(0.0)	6	(2.0)
>N40000	1	(1.3)	4	(5.1)	2	(2.6)	1	(1.4)	8	(2.6)
<N5000	1	(1.3)	2	(2.6)	2	(2.6)	0	(0)	5	(1.7)
None[+]	8	(10.3)	2	(2.6)	1	(1.3)	2	(2.9)	13	(4.3)
Missing	4	(5.1)	0	(0.0)	0	(0.0)	0	(0.0)	4	(1.3)
Total	**78**	**(100.0)**	**78**	**(100.0)**	**76**	**(100.0)**	**70**	**(100.0)**	**302**	**(100.0)**

<u>Notes</u>: [+] – Unemployed and without income

Appendix X: Mouth prevalence of Developmental Defects of Enamel in primary teeth of 4 year-old participants, by area and gender (n=302).

Developmental Defects of Enamel	**Area 1 (Urban, Higher F) No. (%)**	**Area 2 (Rural, Higher F) No. (%)**	**Area 3 (Urban, Lower F) No. (%)**	**Area 4 (Rural, Lower F) No. (%)**	**All areas No. (%)**	**p value***
Male						
Present (DDE > 0)	34 (43.6)	35 (44.9)	38 (50.0)	16 (22.9)	123 (40.7)	<0.001
Absent (DDE = 0)	0 (0)	5 (6.4)	8 (10.5)	19 (27.1)	32 (10.6)	
Total	**34 (43.6)**	**40 (51.3)**	**46 (60.5)**	**35 (50.0)**	**155 (51.3)**	
Female						
Present (DDE > 0)	39 (50.0)	31 (39.7)	22 (28.9)	20 (28.6)	112 (37.1)	0.01
Absent (DDE = 0)	5 (6.4)	7 (9.0)	8 (10.6)	15 (21.4)	35 (11.6)	
Total	**44 (56.4)**	**38 (48.7)**	**30 (39.5)**	**35 (50.0)**	**147 (48.7)**	
All						
Present (DDE > 0)	73 (93.6)	66 (84.6)	60 (78.9)	36 (51.4)	235 (77.8)	<0.001
Absent (DDE = 0)	5 (6.4)	12 (15.4)	16 (21.1)	34 (48.6)	67 (22.2)	
Overall Total	**78 (100.0)**	**78 100.0)**	**76 (100.0)**	**70 (100.0)**	**302 (100.0)**	

*Notes: Between males and females ($p^*=0.51$); * - Chi-square*

Appendix Y: No. of 4 year old children (% of total) with Developmental Defects of Enamel in the primary dentition by area and type of defect (n=302[1]).

Types of Developmental Defects Enamel	Area 1 (Urban, Higher F) (n=78) No. (%)	Area 2 (Rural, Higher F) (n=78) No. (%)	Area 3 (Urban, Lower F) (n=76) No. (%)	Area 4 (Rural, Lower F) (n=70) No. (%)	All Areas (n=302) No. (%)
Demarcated Opacities	10 (12.8)	28 (35.9)	25 (32.9)	1 (1.4)	64 (21.2)
Diffuse Opacities	73 (93.6)	38 (48.7)	32 (42.1)	20 (28.2)	163 (54.0)
Hypoplasia	6 (7.7)	3 (3.9)	9 (11.8)	5 (7.2)	23 (7.6)
Other defects	0 (0)	32 (41.0)	30 (39.5)	12 (18.1)	74 (24.5)
Demarcated and diffuse opacities	0 (0)	1 (1.3)	2 (2.6)	0 (0)	3 (1.0)
Demarcated opacities and hypoplasia	1 (1.3)	1 (1.3)	1 (1.3)	0 (0)	3 (1.0)
Diffuse opacities and hypoplasia	14 (17.9)	5 (10.5)	8 (10.5)	5 (7.1)	32 (10.6)
Demarcated & Diffuse opacities and hypoplasia)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Notes: [1] – Multiple categories were recorded for some children

Appendix Z: Mean (SD) and median (minimum, maximum) number of primary teeth in 4 year-old participants affected by Developmental Defects of Enamel by area and gender (n=302).

No. of teeth affected	Area 1 (Urban, Higher F)	Area 2 (Rural, Higher F)	Area 3 (Urban, Lower F)	Area 4 (Rural, Lower F)	All areas	p value*	Tukey Post-hoc
Male (n) Mean (SD) Median (Min, Max)	34 6.82 (3.00) 6.50 (0, 10)	40 5.03 (3.38) 5.00 (0, 10)	46 3.74 (2.89) 3.50 (0, 10)	35 1.09 (1.72) 0 (0, 7)	155 4.15 (3.45) 4.00 (0, 10)	<0.001	1v2(p=0.04);1v3(p<0.001); 2v4(p<0.001);1v4(p<0.001); 3v4(p<0.001)
Female (n) Mean (SD) Median (Min, Max)	44 5.61 (3.36) 5.50 (0, 10)	38 4.24 (3.11) 4.00 (0, 10)	30 3.27 (2.83) 3.00 (0, 10)	35 1.69 (1.95) 2.00 (0, 8)	147 3.84 (3.23) 4.00 (0, 10)	<0.001	1v3(p=0.01); 2v4(p=0.001); 1v4(p<0.001)
All (n) Mean (SD) Median (Min, Max)	78 6.14 (3.25) 6.00 (1, 10)	78 4.64 (3.25) 4.00 (1, 10)	76 3.55 (2.85) 3.00 (0, 10)	70 1.39 (1.85) 1.00 (0, 8)	302 4.00 (3.33) 4.00 (0, 10)	<0.001	1v2(p=0.01); 1v3(p<0.001); 2v4(p<0.001);1v4(p<0.001); 3v4(p<0.001)

Notes: Between males and females (p=0.43); * *- One way ANOVA*

Appendix AA – Frequency distribution of types of Developmental Defects of Enamel on the primary index teeth of 4 year-old participants.

Types of Developmental Defects Enamel	No.	% of defects	% of index teeth examined
Demarcated Opacities	122	10.1	4.1
Diffuse Opacities	696	57.6	23.3
Hypoplasia	41	3.4	1.4
Other defects	267	22.1	8.9
Demarcated and diffuse opacities	5	0.4	0.2
Demarcated opacities and hypoplasia	7	0.6	0.3
Diffuse opacities and hypoplasia	64	5.3	2.1
Demarcated, Diffuse & Hypoplasia,	0	0	0
Total with defects	**1202**	**100.0**	**40.3**
Could not be recorded[1]	6	N/A	0.2
No defects	1777	N/A	59.5
Overall Total	**2985**	**N/A**	**100.0**

Notes: [1] 6 index teeth could not be recorded because buccal surfaces were not available due to accumulation of debris and dental caries

Appendix AB – Mouth prevalence of Dental Fluorosis in primary teeth of 4 year-olds by area and gender using Dean's index (n=302).

Dental fluorosis	**Area 1 (Urban, Higher F) No. (%)**	**Area 2 (Rural, Higher F) No. (%)**	**Area 3 (Urban, Lower F) No. (%)**	**Area 4 (Rural, Lower F) No. (%)**	**All areas No. (%)**	**p value***
Male						
Present (TFI > 0)	4 (5.1)	6 (7.7)	0 (0)	0 (0)	8 (2.6)	0.001
Absent (TFI = 0)	30 (38.5)	34 (43.6)	46 (60.5)	35 (50.0)	147 (48.7)	
Total	**34 (43.6)**	**40 (51.3)**	**46 (60.5)**	**35 (50.0)**	**155 (51.3)**	
Female						
Present (TFI > 0)	8 (10.3)	3 (3.8)	0 (0)	0 (0)	11 (3.7)	0.001
Absent (TFI = 0)	36 (46.1)	35 (44.9)	30 (39.5)	35 (50.0)	136 (45.0)	
Total	**44 (56.4)**	**38 (48.7)**	**30 (39.5)**	**35 (50.0)**	**147 (48.7)**	
All						
Present (TFI > 0)	12 (15.4)	9 (11.5)	0 (0)	0 (0)	21 (7.0)	p<0.001
Absent (TFI = 0)	66 (84.6)	69 (88.5)	76 (100.0)	70 (100.0)	281 (93.0)	
Total	**78 (100.0)**	**78 (100.0)**	**76 (100.0)**	**70 (100.0)**	**302 (100.0)**	

*Notes: Between males and females (p^*=0.45); * - Chi-square*

Appendix AC – Mouth prevalence of Dental Fluorosis in primary teeth of 4 year-old participants by area and gender using TF index (n=302).

Dental fluorosis	Area 1 (Urban, Higher F) No. (%)	Area 2 (Rural, Higher F) No. (%)	Area 3 (Urban, Lower F) No. (%)	Area 4 (Rural, Lower F) No. (%)	All areas No. (%)	p value*
Male						
Present (TFI > 0)	3 (3.9)	5 (6.3)	0 (0)	0 (0)	8 (2.6)	0.01
Absent (TFI = 0)	31 (39.7)	35 (44.9)	46 (60.5)	35 (50.0)	147 (48.7)	
Total	**34 (43.6)**	**40 (51.2)**	**46 (60.5)**	**35 (50.0)**	**155 (51.3)**	
Female						
Present (TFI > 0)	6 (7.7)	3 (3.9)	0 (0)	0 (0)	9 (3.0)	0.01
Absent (TFI = 0)	38 (48.7)	35 (44.9)	30 (39.5)	35 (50.0)	138 (45.7)	
Total	**44 (56.4)**	**38 (48.8)**	**30 (39.5)**	**35 (50.0)**	**147 (48.7)**	
All						
Present (TFI > 0)	9 (11.5)	8 (10.3)	0 (0)	0 (0)	17 (5.6)	<0.001
Absent (TFI = 0)	69 (88.5)	70 (89.7)	76 (100.0)	70 (100.0)	285 (94.4)	
Total	**78 (100.0)**	**78 (100.0)**	**76 (100.0)**	**70 (100.0)**	**302 (100.0)**	

*Notes: Between males and females (p^*=0.81); * - Chi-square*

Appendix AD – Mouth prevalence of Dental Fluorosis using digital photographic imaging of primary upper central incisors among 4 year-old participants by area and gender (n=302).

Dental fluorosis	Area 1 (Urban, Higher F) No. (%)	Area 2 (Rural, Higher F) No. (%)	Area 3 (Urban, Lower F) No. (%)	Area 4 (Rural, Lower F) No. (%)	All areas No. (%)	p value*
Male						
Present (TFI > 0)	1 (1.3)	0 (0)	1 (1.3)	0 (0)	2 (0.7)	0.44
Absent (TFI = 0)	33 (42.3)	40 (51.3)	45 (59.2)	35 (50.0)	153 (50.7)	
Total	**34 (43.6)**	**40 (51.3)**	**46 (60.5)**	**35 (50.0)**	**155 (51.4)**	
Female						
Present (TFI > 0)	3 (3.8)	1 (1.3)	0 (0)	1 (1.4)	5 (1.6)	0.34
Absent (TFI = 0)	41 (52.6)	37 (47.4)	30 (39.5)	34 (48.6)	142 (47.0)	
Total	**44 (56.4)**	**38 (48.7)**	**30 (39.5)**	**35 (50.0)**	**147 (48.6)**	
All						
Present (TFI > 0)	4 (5.1)	1 (1.3)	1 (1.3)	1 (1.4)	7 (2.3)	0.31
Absent (TFI = 0)	74 (94.9)	77 (98.7)	75 (98.7)	69 (98.6)	295 (97.7)	
Total	**78 (100.0)**	**78 (100.0)**	**76 (100.0)**	**70 (100.0)**	**302 (100.0)**	

*Notes: Between males and females (p^*=0.27); * - Chi-square*

Appendix AE – Mean (SD) and median (minimum, maximum) number of primary teeth in 4 year-old participants affected by dental fluorosis, by area and gender (n=302).

No. of teeth affected by dental fluorosis	Area 1 (Urban, Higher F)	Area 2 (Rural, Higher F)	Area 3 (Urban, Lower F)	Area 4 (Rural, Lower F)	All areas	p value*	Tukey Post-hoc
Male (n) Mean (SD) Median (Min, Max)	34 0.47 (1.85) 0 (0, 10)	40 0.65 (2.03) 0 (0, 9)	46 0 (0) 0 (0)	35 0 (0) 0 (0)	155 0.27 (1.36) 0 (0, 10)	0.70	
Female (n) Mean (SD) Median (Min, Max)	44 0.34 (0.94) 0 (0, 4)	38 0.42 (1.98) 0 (0, 12)	30 0 (0) 0 (0)	35 0 (0) 0 (0)	147 0.21 (1.14) 0 (0, 12)	0.25	
All (n) Mean (SD) Median (Min, Max)	78 0.40 (1.40) 0 (0, 10)	78 0.54 (2.00) 0 (0, 12)	76 0 (0) 0 (0)	70 0 (0) 0 (0)	302 0.24 (1.26) 0 (0, 12)	0.01	2v3(p=0.04); 2v4(p=0.04)

*Notes: Between males and females (p^*=0.64); * - One way Anova*

Appendix AF – Mouth prevalence of caries experience in primary teeth of 4 year-old participants, by area and gender (n=302).

Caries experience	**Area 1 (Urban, Higher F) No. (%)**	**Area 2 (Rural, Higher F) No. (%)**	**Area 3 (Urban, Lower F) No. (%)**	**Area 4 (Rural, Lower F) No. (%)**	**All areas No. (%)**	**p value***
Male						
Present	9 (11.5)	1 (1.3)	7 (9.2)	2 (2.9)	19 (6.3)	0.01
Absent	25 (32.1)	39 (50.0)	39 (51.3)	33 (47.1)	136 (45.0)	
Total	**34 (43.6)**	**40 (51.3)**	**46 (60.5)**	**35 (50.0)**	**155 (51.3)**	
Female						
Present	5 (6.4)	2 (2.6)	3 (4.0)	3 (4.3)	13 (4.3)	0.78
Absent	39 (50.0)	36 (46.1)	27 (35.5)	32 (45.7)	134 (44.4)	
Total	**44 (56.4)**	**38 (48.7)**	**30 (39.5)**	**35 (50.0)**	**147 (48.7)**	
All						
Present	14 (17.9)	3 (3.8)	10 (13.2)	5 (7.1)	32 (10.6)	0.02
Absent	64 (82.1)	75 (96.2)	66 (86.8)	65 (92.9)	270 (89.4)	
Total	**78 (100.0)**	**78 (100.0)**	**76 (100.0)**	**70 (100.0)**	**302 (100.0)**	

*Notes: Between males and females ($p^*=0.36$); * - Chi-square*

Appendix AG – Mean (SD) and median (minimum, maximum) number of primary teeth in 4 year-old participants affected by Dental Caries, by area and gender (n=302).

No. of teeth affected by dental caries	Area 1 (Urban, Higher F)	Area 2 (Rural, Higher F)	Area 3 (Urban, Lower F)	Area 4 (Rural, Lower F)	All areas	p value*	Tukey Post-hoc
Male (n) Mean (SD) Median (Min, Max)	34 0.88 (1.90) 0 (0, 8)	40 0.05 (0.32) 0 (0, 2)	46 0.48 (1.33) 0 (0, 6)	35 0.09 (0.37) 0 (0, 2)	155 0.37 (1.21) 0 (0, 8)	0.01	1v2(p=0.02); 1v4(p=0.03)
Female (n) Mean (SD) Median (Min, Max)	44 0.27 (0.85) 0 (0, 4)	38 0.08 (0.36) 0 (0, 2)	30 0.13 (0.43) 0 (0, 2)	35 0.37 (1.50) 0 (0, 8)	147 0.22 (0.90) 0 (0, 8)	0.51	
All (n) Mean (SD) Median (Min, Max)	78 0.54 (1.43) 0 (0, 8)	78 0.06 (0.34) 0 (0, 2)	76 0.34 (1.08) 0 (0, 6)	70 0.23 (1.09) 0 (0, 8)	302 0.29 (1.07) 0 (0, 8)	0.04	1v2(p=0.03)

Notes: Between males and females (p^*=0.22) - One way ANOVA*

Appendix AH – Mean (±SD) age and gender of 322 8-year-old study participants by area.

Bio-data	**Area 1 (Urban, Higher) (n=81)**	**Area 2 (Rural, Higher) (n=79)**	**Area 3 (Urban, Lower) (n=79)**	**Area 4 (Rural, Lower) (n=83)**	**Total (n=322)**	**p value**
Age (years)						
Mean(SD)	8.49 (0.31)	8.58 (0.29)	8.52 (0.31)	8.58 (0.26)	8.5 (0.30)	0.11*
Minimum	8.0	8.0	8.01	8.08	8.00	
Maximum	8.97	8.95	8.94	8.95	8.97	
Gender						
Male No. (%)	39 (48.1)	27 (34.2)	40 (50.6)	49 (59.0)	155 (48.1)	0.02+
Female No. (%)	42 (51.9)	52 (65.8)	39 (49.4)	34 (41.0)	167 (51.9)	
Total	**81 (100.0)**	**79 (100.0)**	**79 (100.0)**	**83 (100.0)**	**322(100.0)**	

*Notes: * - One way Anova; + - chi-square*

Appendix AI – Duration of residence of 8 year-olds in present location by area and gender (n = 322).

Duration of residence in Area	**Area 1 (Urban, Higher F) No. (%)**	**Area 2 (Rural, Higher F) No. (%)**	**Area 3 (Urban, Lower F) No. (%)**	**Area 4 (Rural, Lower F) No. (%)**	**All areas No. (%)**	**p value***
Male						
From birth	24 (29.6)	22 (27.9)	29 (36.7)	33 (39.8)	108 (33.6)	0.35
After birth	15 (18.5)	5 (6.3)	11 (13.9)	16 (19.3)	47 (14.6)	
Total	**39 (48.1)**	**27 (34.2)**	**40 (50.6)**	**49 (59.1)**	**155 (48.2)**	
Female						
From birth	22 (27.2)	49 (62.0)	27 (23.7)	27 (32.5)	125 (38.8)	<0.001
After birth	20 (24.7)	3 (3.8)	12 (15.8)	7 (8.4)	42 (13.0)	
Total	**42 (51.9)**	**52 (65.8)**	**39 (39.5)**	**34 (40.9)**	**167 (48.7)**	
All						
From birth	46 (56.8)	71 (89.8)	56 (59.2)	60 (72.3)	233 (72.4)	<0.001
After birth	35 (43.2)	8 (10.2)	23 (40.8)	23 (27.7)	89 (27.6)	
Total	**81 (100.0)**	**79 (100.0)**	**79 (100.0)**	**83 (100.0)**	**322 (100.0)**	

*Notes: Between males and females (p^*=0.32); * - Chi-square*

Appendix AJ – Social characteristics (Level of education, occupation and income) of parents or legal guardians of 8 year old study participants by area (n=322).

Social characteristics	Area 1 (Urban, Higher F)		Area 2 (Rural, Higher F)		Area 3 (Urban, Lower F)		Area 4 (Rural, Lower F)		Total	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)
Level of education										
Never went to school	21	(25.9)	9	(11.4)	18	(22.8)	24	(28.9)	72	(22.4)
Primary school	28	(34.6)	37	(46.8)	27	(34.1)	36	(43.4)	128	(39.8)
Koranic school	2	(2.5)	0	(0.0)	1	(1.3)	0	(0.0)	3	(0.9)
Secondary school	23	(28.4)	22	(27.8)	29	(36.7)	21	(25.3)	95	(29.5)
Vocational school	0	(0.0)	1	(1.3)	1	(1.3)	0	(0.0)	2	(0.6)
Polytechnic/University	7	(8.6)	10	(12.7)	3	(3.8)	2	(2.4)	22	(6.8)
Total	**81**	**(100.0)**	**79**	**(100.0)**	**79**	**(100.0)**	**83**	**(100.0)**	**322**	**(100.0)**
Occupation										
Business woman/man	22	(27.2)	1	(1.3)	1	(1.3)	3	(3.6)	27	(8.4)
Unemployed/housewife	6	(7.4)	3	(3.8)	1	(1.3)	3	(3.6)	13	(4.0)
Trader	35	(43.2)	42	(53.1)	55	(69.6)	56	(67.5)	188	(58.4)
Artisans/farmer	13	(16.0)	23	(29.1)	15	(19.0)	19	(22.9)	70	(21.7)
Civil servants	1	(1.2)	8	(10.1)	5	(6.3)	2	(2.4)	16	(5.1)
Bankers/engineers	1	(1.2)	1	(1.3)	2	(2.5)	0	(0.0)	4	(1.2)
Domestic staff	3	(3.7)	1	(1.3)	0	(0.0)	0	(0.0)	4	(1.2)
Total	**81**	**(100.0)**	**79**	**(100.0)**	**79**	**(100.0)**	**83**	**(100.0)**	**322**	**(100.0)**
Monthly Income										
N5,000 - N10000	42	(51.9)	41	(51.9)	52	(65.8)	56	(67.5)	191	(59.3)
N11000 - N20000	15	(18.5)	7	(8.9)	10	(12.7)	19	(22.9)	51	(15.8)
N21000 – N30000	9	(11.1)	10	(12.7)	5	(6.3)	5	(6.0)	29	(9.0)
N31000 – N40000	5	(6.2)	6	(7.6)	3	(3.8)	0	(0.0)	14	(4.3)
>N40000	4	(4.9)	6	(7.6)	7	(8.9)	0	(0.0)	17	(5.2)
<N5000	1	(1.2)	6	(7.6)	1	(1.3)	0	(0.0)	8	(2.5)
None[1]	3	(3.7)	3	(3.8)	1	(1.3)	3	(3.6)	10	(3.1)
Missing	2	(2.5)	0	(0.0)	0	(0.0)	0	(0.0)	2	(0.6)
Total	**81**	**(100.0)**	**79**	**(100.0)**	**79**	**(100.0)**	**83**	**(100.0)**	**322**	**(100.0)**

Notes: [1] - Unemployed and without income

Appendix AK – Mouth prevalence of Developmental Defects of Enamel in primary teeth of 8 year-old participants, by area and gender (n=322).

Developmental Defects of Enamel	**Area 1 (Urban, Higher F) No. (%)**	**Area 2 (Rural, Higher F) No. (%)**	**Area 3 (Urban, Lower F) No. (%)**	**Area 4 (Rural; Lower F) No. (%)**	**All areas No. (%)**	**p value***
Male						
Present (DDE > 0)	35 (43.2)	14 (17.7)	35 (44.3)	20 (24.1)	104 (32.3)	<0.001
Absent (DDE = 0)	4 (4.9)	13 (16.5)	5 (6.3)	29 (34.9)	51 (15.8)	
Total	**39 (48.1)**	**27 (34.2)**	**40 (50.6)**	**49 (59.0)**	**155 (48.1)**	
Female						
Present (DDE > 0)	27 (33.4)	34 (43.0)	29 (36.7)	15 (18.1)	105 (32.6)	0.06
Absent (DDE = 0)	15 (18.5)	18 (22.8)	10 (12.7)	19 (22.9)	62 (19.3)	
Total	**42 (51.9)**	**52 (65.8)**	**39 (49.4)**	**34 (41.0)**	**167 (51.9)**	
All						
Present (DDE > 0)	62 (76.5)	48 (60.8)	64 (81.0)	35 (42.2)	209 (64.9)	<0.001
Absent (DDE = 0)	19 (23.5)	31 (39.2)	15 (19.0)	48 (57.8)	113 (35.1)	
Total	**81 (100.0)**	**79 (100.0)**	**79 (100.0)**	**83 (100.0)**	**322 (100.0)**	

*Notes: Between males and females (p^*=0.25); * - Chi-square*

Appendix AL– No. of 8 year old children (% of total) with Developmental Defects of Enamel in the primary dentition by area and type of defect (n=322[1]).

Types of Developmental Defects Enamel	Area 1 (Urban, Higher F) (n=81) No. (%)	Area 2 (Rural, Higher F) (n=79) No. (%)	Area 3 (Urban, Lower F) (n=79) No. (%)	Area 4 (Rural, Lower F) (n=83) No. (%)	All Areas (n=322) No. (%)
Demarcated Opacities	3 (3.7)	8 (10.1)	16 (20.3)	8 (9.6)	35 (10.9)
Diffuse Opacities	59 (72.8)	32 (40.5)	24 (30.4)	9 (10.8)	124 (38.5)
Hypoplasia	4 (4.9)	0 (0)	1 (1.3)	2 (2.4)	7 (2.2)
Other defects	0 (0)	17 (21.5)	44 (55.7)	17 (20.5)	78 (24.2)
Demarcated and diffuse opacities	0 (0)	0 (0)	4 (5.1)	4 (4.8)	8 (2.5)
Demarcated opacities and hypoplasia	0 (0)	1 (1.3)	0 (0)	0 (0)	1 (0.3)
Diffuse opacities and hypoplasia	9 (11.1)	3 (3.8)	4 (5.1)	3 (3.6)	19 (5.9)
Demarcated & Diffuse opacities and hypoplasia	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Notes: [1] – Multiple categories were recorded for some children

Appendix AM – Mean (SD) and median (minimum, maximum) number of primary teeth in 8 year-old participants affected by Developmental Defects of Enamel by area and gender (n=322).

Developmental Defects of Enamel (No. of teeth affected)	Area 1 (Urban, Higher F)	Area 2 (Rural, Higher F)	Area 3 (Urban, Lower F)	Area 4 (Rural, Lower F)	All areas	p value*	Tukey Post-hoc
Male (n) Mean (SD) Median (Min, Max)	39 3.31(2.13) 3.00(0, 10)	27 1.67(1.90) 1.00(0, 6)	40 2.55(1.48) 2.50(0, 10)	49 0.90(1.30) 0(0, 5)	155 2.06(1.93) 2.00(0, 10)	<0.001	1v2(p=0.001); 1v3(p<0.001); 1v4(p<0.001); 3v4(p<0.001)
Female (n) Mean (SD) Median (Min, Max)	42 2.48(2.35) 3.00(0, 10)	52 2.19(1.85) 2.00(0, 6)	39 2.08(1.56) 2.00(0, 4)	34 1.00(1.28) 1.00(0, 4)	167 1.99(1.89) 2.00(0, 10)	0.004	2v4(p=0.02); 1v4(p=0.003)
All (n) Mean (SD) Median (Min, Max)	81 2.88(2.27) 3.00(1, 10)	79 2.01(1.87) 2.00(1, 6)	79 2.32(1.53) 2.00(0, 5)	83 0.94(1.28) 0.00(0, 5)	322 2.03(1.91) 2.00(0, 10)	<0.001	1v2(p=0.01); 2v4(p=0.001) 1v4(p<0.001); 3v4(p<0.001)

Notes: Between males and females (p=0.74); * - One way Anova*

Appendix AN – Frequency distribution of types of Developmental Defects of Enamel in index teeth of the primary dentition of 8 year-old participants (n=322).

Types of Developmental Defects Enamel	No.	% of defects	% of index teeth examined
Demarcated Opacities	41	6.5	2.8
Diffuse Opacities	337	53.2	23.0
Hypoplasia	13	2.1	0.9
Other defects	192	30.3	13.1
Demarcated and diffuse opacities	10	1.6	0.7
Demarcated opacities and hypoplasia	1	0.1	0.1
Diffuse opacities and hypoplasia	39	6.2	2.6
Demarcated, Diffuse & Hypoplasia,	0	0	0
Total with defects	**633**	**100.0**	**43.1**
Could not be recorded[1]	20	N/A	1.4
No defects	814	N/A	55.5
Overall Total	**1467**	**N/A**	**100.0**

Notes: [1] 20 index teeth could not be recorded because buccal surfaces were not available due to debris and dental caries.

Appendix AO – Mouth prevalence of dental fluorosis in primary teeth of 8 year-old participants, by area and gender (n=322) using TF Index.

Dental fluorosis	Area 1 (Urban, Higher F) No. (%)	Area 2 (Rural, Higher F) No. (%)	Area 3 (Urban, Lower F) No. (%)	Area 4 (Rural, Lower F) No. (%)	All areas No. (%)	p value*
Male						
Present (TFI > 0)	6 (7.4)	8 (10.1)	0 (0)	0 (0)	14 (4.3)	<0.001
Absent (TFI = 0)	33 (40.8)	19 (24.1)	40 (50.6)	49 (59.0)	141 (43.8)	
Total	**39 (48.2)**	**27 (34.2)**	**40 (50.6)**	**49 (59.0)**	**155 (48.1)**	
Female						
Present (TFI > 0)	4 (4.9)	9 (11.4)	0 (0)	3 (3.6)	16 (5.0)	0.05
Absent (TFI = 0)	38 (46.9)	43 (54.4)	39 (49.4)	31 (37.4)	151 (46.9)	
Total	**42 (51.8)**	**52 (65.8)**	**39 (49.4)**	**34 (41.0)**	**167 (51.9)**	
All						
Present (TFI > 0)	10 (12.3)	17 (21.5)	0 (0)	3 (3.6)	30 (9.3)	<0.001
Absent (TFI = 0)	71 (87.7)	62 (78.5)	79 (100.0)	80 (96.4)	292 (90.7)	
Total	**81 (100.0)**	**79 (100.0)**	**79 (100.0)**	**83 (100.0)**	**322 (100.0)**	

Notes: Between males and females (p=1.00); * - Chi-square*

Appendix AP – Mean (SD) and median (minimum, maximum) number of primary teeth in 8 year-old participants affected by dental fluorosis, by area and gender (n=322).

Dental fluorosis (No. of teeth affected)	Area 1 (Urban, Higher F)	Area 2 (Rural, Higher F)	Area 3 (Urban, Lower F)	Area 4 (Rural, Lower F)	All areas	p value*	Tukey Post-hoc
Male (n) Mean (SD) Median (Min, Max)	39 0.77 (2.08) 0 (0, 10)	27 1.59 (3.31) 0 (0, 14)	40 0 (0) 0 (0)	49 0 (0) 0 (0)	155 0.47 (1.81) 0 (0, 14)	<0.001	2v3(p=0.002); 2v4(p=0.001)
Female (n) Mean (SD) Median (Min, Max)	42 0.52 (1.88) 0 (0, 10)	52 0.83 (1.97) 0 (0, 8)	39 0 (0) 0 (0)	34 0.21 (0.73) 0 (0, 3)	167 0.43 (1.51) 0 (0, 10)	0.05	
All (n) Mean (SD) Median (Min, Max)	81 0.64(1.97) 0 (0, 10)	79 1.09 (2.51) 0 (0, 14)	79 0 (0) 0 (0)	83 0.08 (0.47) 0 (0, 3)	322 0.45 (1.66) 0 (0, 14)	<0.001	2v3(p<0.001); 2v4(p=0.001)

Notes: Between males and females (p=0.83); * - One way ANOVA*

Appendix AQ – Mouth prevalence of caries experience in primary teeth of 8 year-old participants, by area and gender (n=322).

Dental caries	**Area 1 (Urban, Higher F) No. (%)**	**Area 2 (Rural, Higher F) No. (%)**	**Area 3 (Urban, Lower) No. (%)**	**Area 4 (Rural, Lower) No. (%)**	**All areas No. (%)**	**p value***
Male						
Present	14 (17.3)	4 (5.1)	7 (8.9)	1 (1.2)	26 (8.1)	<0.001
Absent	25 (30.9)	23 (29.1)	33 (41.8)	48 (57.8)	129 (40.1)	
Total	**39 (48.2)**	**27 (34.2)**	**40 (50.7)**	**49 (59.0)**	**155 (48.2)**	
Female						
Present	15 (18.5)	3 (3.8)	9 (11.4)	1 (1.2)	28 (8.7)	<0.001
Absent	27 (33.3)	49 (62.0)	30 (37.9)	33 (39.8)	139 (43.1)	
Total	**42 (100.0)**	**52 (65.8)**	**39 (49.3)**	**34 (41.0)**	**167 (51.8)**	
All						
Present	29 (35.8)	7 (8.9)	16 (20.3)	2 (2.4)	54 (16.8)	<0.001
Absent	52 (64.2)	72 (91.1)	66 (79.7)	81 (97.6)	268 (83.2)	
Total	**81 (100.0)**	**79 (100.0)**	**79 (100.0)**	**83 (100.0)**	**322 (100.0)**	

*Notes: Between males and females (p^*=1.00); * - Chi-square*

Appendix AR – Mean (SD) and median (minimum, maximum) number of primary teeth in 8 year-old participants affected by caries experience, by area and gender (n=322).

Dental fluorosis (No. of teeth affected)	Area 1 (Urban, Higher F)	Area 2 (Rural, Higher F)	Area 3 (Urban, Lower F)	Area 4 (Rural, Lower F)	All areas	p value*	Tukey Post-hoc
Male (n) Mean (SD) Median (Min, Max)	39 0.87 (1.49) 0 (0, 6)	27 0.26 (0.81) 0 (0, 4)	40 0.35(0.86) 0 (0, 4)	49 0.08 (0.57) 0 (0, 4)	155 0.38 (1.02) 0 (0, 6)	0.003	1v4(p<0.01)
Female (n) Mean (SD) Median (Min, Max)	42 1.02 (1.87) 0 (0, 4)	52 0.10 (0.45) 0 (0, 3)	39 0.72 (1.61) 0 (0, 7)	34 0.06 (0.34) 0 (0, 2)	167 0.47 (1.31) 0 (0, 10)	0.001	1v2(p=0.003); 1v4(p=0.01)
All (n) Mean (SD) Median (Min, Max)	81 0.95 (1.69) 0 (0, 10)	79 0.15 (0.60) 0 (0, 4)	79 0.53 (1.29) 0 (0, 7)	83 0.07 (0.49) 0 (0, 4)	322 0.43(1.18) 0 (0, 10)	<0.001	1v2(p<0.001);1v4(p<0.001)

Notes: Between males and females (p=0.51); * - One way ANOVA*

Appendix AS – Mouth prevalence of Developmental Defects of Enamel in permanent teeth of 8 year-old participants, by area and gender (n=322).

Developmental Defects of Enamel	**Area 1 (Urban, Higher F) No. (%)**	**Area 2 (Rural, Higher F) No. (%)**	**Area 3 (Urban, Lower F) No. (%)**	**Area 4 (Rural, Lower F No. (%)**	**All areas No. (%)**	**p value***
Male						
Present (DDE > 0)	32 (39.5)	21 (26.6)	26 (32.9)	27 (32.5)	106 (32.9)	0.03
Absent (DDE = 0)	7 (8.6)	6 (7.6)	14 (17.7)	22 (26.5)	49 (15.2)	
Total	**39 (48.1)**	**27 (34.2)**	**40 (50.6)**	**49 (59.0)**	**155 (48.1)**	
Female						
Present (DDE > 0)	27 (33.3)	46 (58.2)	21 (26.6)	16 (19.3)	110 (34.2)	<0.001
Absent (DDE = 0)	15 (18.6)	6 (7.6)	18 (22.8)	18 (21.7)	57 (17.7)	
Total	**42 (51.9)**	**52 (65.8)**	**39 (49.4)**	**34 (41.0)**	**167 (51.9)**	
All						
Present (DDE > 0)	59 (72.8)	67 (84.8)	47 (59.5)	43 (51.8)	216 (67.1)	<0.001
Absent (DDE = 0)	22 (27.2)	12 (15.2)	32 (40.5)	40 (48.2)	106 (32.9)	
Total	**81 (100.0)**	**79 (100.0)**	**79 (100.0)**	**83 (100.0)**	**322 (100.0)**	

*Notes: Between males and females (p^*=0.64); * - Chi-square*

Appendix AT – Mean (SD) and median (minimum, maximum) number of permanent teeth in 8 year-old participants affected by Developmental Defects of Enamel by area and gender (n=322).

Developmental Defects of Enamel (No. of teeth affected)	Area 1 (Urban, Higher F)	Area 2 (Rural, Higher F)	Area 3 (Urban, Lower F)	Area 4 (Rural, Lower F)	All areas	p value*	Tukey Post-hoc
Male (n) Mean (SD) Median (Min, Max)	39 2.95 (1.86) 3.00 (0, 6)	27 3.41 (2.32) 4.00 (0, 6)	40 2.18 (2.31) 2.00 (0, 10)	49 1.04 (1.26) 1.00 (0, 6)	155 2.23 (2.11) 2.00 (0, 10)	<0.001	2v4(p<0.001); 1v4(p<0.001); 3v4(p=0.03)
Female (n) Mean (SD) Median (Min, Max)	42 2.05 (2.02) 2.00 (0, 6)	52 4.08 (2.08) 4.00 (0, 6)	39 1.72 (2.19) 1.00 (0, 8)	34 1.53 (1.99) 0 (0, 6)	167 2.50 (2.32) 2.00 (0, 8)	<0.001	1v2(p<0.001); 2v3(p<0.001) 2v4(p<0.001)
All (n) Mean (SD) Median (Min, Max)	81 2.48 (1.99) 2.00 (0, 6)	79 3.85 (2.17) 4.00 (0, 6)	79 1.95 (2.25) 2.00 (0, 10)	83 1.24 (1.61) 1.00 (0, 6)	322 2.37 (2.22) 2.00 (0, 10)	<0.001	1v2(p<0.001); 2v3(p<0.001) 2v4(p<0.001); 1v4(p=0.001)

Notes: Between males and females (p=0.27); * - One way Anova*

Appendix AU – Frequency distribution of types of Developmental Defects of Enamel in index teeth of the permanent dentition of 8 year-old participants.

Types of Developmental Defects Enamel	No.	% of defects	% of index teeth examined
Demarcated Opacities	40	5.7	2.4
Diffuse Opacities	461	65.3	27.8
Hypoplasia	35	5.0	2.1
Other defects	59	8.4	3.6
Demarcated and diffuse opacities	16	2.3	1.0
Demarcated opacities and hypoplasia	5	0.7	0.3
Diffuse opacities and hypoplasia	84	11.9	5.1
Demarcated, Diffuse & Hypoplasia,	6	0.8	0.4
Total with defects	**706**	**100.0**	**43.1**
Could not be recorded[1]	36	N/A	1.4
No defects	913	N/A	55.5
Overall Total	**1655**	**N/A**	**100.0**

Notes: [1] 36 index teeth could not be recorded because buccal surfaces were not available due to debris and dental caries

Appendix AV – Mouth prevalence of Dental Fluorosis in permanent teeth of 8 year-old participants, by area and gender (n=322) using Dean's index.

Dental fluorosis	**Area 1 (Urban, Higher F) No. (%)**	**Area 2 (Rural, Higher F) No. (%)**	**Area 3 (Urban, Lower F) No. (%)**	**Area 4 (Rural, Lower F) No. (%)**	**All areas No. (%)**	**p value***
Male						
Present (TFI > 0)	10 (12.3)	22 (27.9)	2 (2.5)	0 (0)	34 (10.6)	<0.001
Absent (TFI = 0)	29 (35.9)	5 (6.3)	38 (48.1)	49 (59.0)	121 (37.6)	
Total	**39 (48.2)**	**27 (34.2)**	**40 (50.6)**	**49 (59.0)**	**155 (48.2)**	
Female						
Present (TFI > 0)	10 (12.3)	43 (54.4)	2 (2.5)	5 (6.0)	60 (18.6)	<0.001
Absent (TFI = 0)	32 (39.5)	9 (11.4)	37 (46.9)	29 (35.0)	107 (33.2)	
Total	**42 (41.8)**	**52 (65.8)**	**39 (49.4)**	**34 (41.0)**	**167 (51.8)**	
All						
Present (TFI > 0)	20 (24.7)	65 (82.3)	4 (5.1)	5 (6.0)	94 (29.2)	<0.001
Absent (TFI = 0)	61 (75.3)	14 (17.7)	75 (94.9)	78 (94.0)	228 (70.8)	
Total	**81 (100.0)**	**79 (100.0)**	**79 (100.0)**	**83 (100.0)**	**322 (100.0)**	

*Notes: Between males and females ($p^*=0.01$); * - Chi-square*

Appendix AW – Mouth prevalence of dental fluorosis in permanent teeth of 8 year-old participants, by area and gender (n=322) using TF Index.

Dental fluorosis	Area 1 (Urban, Higher F) No. (%)	Area 2 (Rural, Higher F) No. (%)	Area 3 (Urban, Lower F) No. (%)	Area 4 (Rural, Lower F) No. (%)	All areas No. (%)	p value*
Male						
Present (TFI > 0)	10 (12.3)	22 (27.9)	2 (2.5)	1 (1.2)	35 (10.9)	<0.001
Absent (TFI = 0)	29 (35.9)	5 (6.3)	38 (48.1)	48 (57.8)	120 (37.3)	
Total	**39 (48.2)**	**27 (34.2)**	**40 (50.6)**	**49 (59.0)**	**155 (48.2)**	
Female						
Present (TFI > 0)	10 (12.3)	43 (54.4)	2 (2.5)	6 (7.2)	61 (18.9)	<0.001
Absent (TFI = 0)	32 (39.5)	9 (11.4)	37 (46.9)	28 (33.8)	106 (32.9)	
Total	**42 (51.8)**	**52 (100.0)**	**39 (49.4)**	**34 (41.0)**	**167 (51.8)**	
All						
Present (TFI > 0)	20 (24.7)	65 (82.3)	4 (5.1)	7 (8.4)	96 (29.8)	<0.001
Absent (TFI = 0)	61 (75.3)	14 (17.7)	75 (94.9)	76 (91.6)	226 (70.2)	
Total	**81 (100.0)**	**79 (100.0)**	**79 (100.0)**	**83 (100.0)**	**322 (100.0)**	

*Notes: Between males and females ($p^*=0.01$); * - Chi-square*

Appendix AX – Mouth prevalence of Dental Fluorosis using photographic imaging of permanent upper central incisors among 8 year-olds study participants by area and gender (n=322).

Dental fluorosis	Area 1 (Urban, Higher F) No. (%)	Area 2 (Rural, Higher F) No. (%)	Area 3 (Urban, Lower F) No. (%)	Area 4 (Rural, Lower F) No. (%)	All areas No. (%)	p value*
Male						
Present (TFI > 0)	15 (18.5)	15 (19.0)	5 (6.3)	6 (7.2)	41 (12.7)	<0.001
Absent (TFI = 0)	24 (29.7)	12 (15.2)	35 (44.3)	43 (51.8)	114 (35.4)	
Total	**39 (48.2)**	**27 (34.2)**	**40 (50.6)**	**49 (59.0)**	**155 (48.1)**	
Female						
Present (TFI > 0)	15 (18.5)	33 (41.8)	6 (7.6)	8 (9.7)	62 (19.3)	<0.001
Absent (TFI = 0)	27 (33.3)	19 (24.0)	33 (41.8)	26 (31.3)	105 (32.6)	
Total	**42 (51.8)**	**52 (65.8)**	**39 (49.4)**	**34 (41.0)**	**164 (51.9)**	
All						
Present (TFI > 0)	30 (37.0)	48 (60.8)	11 (13.9)	14 (16.9)	103 (32.0)	<0.001
Absent (TFI = 0)	51 (63.0)	31 (39.2)	68 (86.1)	69 (83.1)	219 (68.0)	
Total	**81 (100.0)**	**79 (100.0)**	**79 (100.0)**	**83 (100.0)**	**322 (100.0)**	

*Notes: Between males and females ($p^*=0.04$); * - Chi-square*

Appendix AY – Mean (SD) and median (minimum, maximum) number of permanent teeth in 8 year-old participants affected by dental fluorosis, by area and gender (n=322).

Dental fluorosis (No. of teeth affected)	Area 1 (Urban, Higher F)	Area 2 (Rural, Higher F)	Area 3 (Urban, Lower F)	Area 4 (Rural, Lower F)	All areas	p value*	Tukey Post-hoc
Male (n) Mean (SD) Median (Min, Max)	39 1.21 (2.45) 0 (0, 10)	27 6.26 (4.00) 6.00 (0, 12)	40 0.38 (1.84) 0 (0, 11)	49 0.20 (1.43) 0 (0, 10)	155 1.55 (3.24) 0 (0, 12)	<0.001	1v2(p<0.001); 2v3(p<0.001) 2v4(p<0.001)
Female (n) Mean (SD) Median (Min, Max)	42 1.24 (2.72) 0 (0, 12)	52 6.42 (4.07) 6.00 (0, 14)	39 0.08 (0.35) 0 (0, 2)	34 1.26 (3.03) 0 (0, 12)	167 2.59 (3.96) 0 (0, 14)	<0.001	1v2(p<0.001); 2v3(p<0.001) 2v4(p<0.001)
All (n) Mean (SD) Median (Min, Max)	81 1.22 (2.58) 0 (0, 12)	79 6.37 (4.02) 6.00 (0, 14)	79 0.23 (1.33) 0 (0, 11)	83 0.64 (2.27) 0 (0, 12)	322 2.09 (3.66) 0 (0, 14)	<0.001	1v2(p<0.001); 2v3(p<0.001); 2v4(p<0.001)

*Notes: Between males and females (p^*=0.01); * - One way Anova*

Appendix AZ – Mouth prevalence of caries experience in permanent teeth of 8 year-old participants, by area and gender (n=322).

Dental caries	**Area 1 (Urban, Higher F) No. (%)**	**Area 2 (Rural, Higher F) No. (%)**	**Area 3 (Urban, Lower F) No. (%)**	**Area 4 (Rural, Lower F) No. (%)**	**All areas No. (%)**	**p value***
Male						
Present	6 (7.4)	0 (0)	5 (6.3)	0 (0)	11 (3.4)	0.001
Absent	33 (40.7)	27 (34.2)	35 (44.3)	49 (59.0)	144 (44.7)	
Total	**39 (48.1)**	**27 (34.2)**	**40 (25.8)**	**49 (59.0)**	**155 (48.1)**	
Female						
Present	5 (6.2)	3 (3.8)	5 (6.3)	0 (0)	13 (4.1)	0.05
Absent	37 (45.7)	49 (62.0)	34 (43.1)	34 (41.0)	154 (47.8)	
Total	**42 (51.9)**	**52 (65.8)**	**39 (23.4)**	**34 (41.0)**	**167 (51.9)**	
All						
Present	11 (13.6)	3 (3.8)	10 (12.7)	0 (0)	24 (7.5)	<0.001
Absent	70 (86.4)	76 (96.2)	69 (87.3)	83 (100.0)	298 (92.5)	
Total	**81 (100.0)**	**79 (100.0)**	**79 (100.0)**	**83 (100.0)**	**322 (100.0)**	

*Notes: Between males and females ($p^*=0.84$); * - Chi-square*

Appendix BA - Mean (SD) and median (minimum, maximum) number of permanent teeth in 8 year-old participants affected by caries experience, by area and gender (n=322).

Dental caries (No. of teeth affected)	Area 1 (Urban, Higher F)	Area 2 (Rural, Higher F)	Area 3 (Urban, Lower F)	Area 4 (Rural, Lower F)	All areas	p value*	Tukey Post-hoc
Male (n) Mean (SD) Median (Min, Max)	39 0.33 (0.84) 0 (0, 3)	27 0 (0) 0 (0)	40 0.23 (0.73) 0 (0, 4)	49 0 (0) 0 (0)	155 0.14 (0.58) 0 (0, 4)	0.02	1v4(p=0.03)
Female (n) Mean (SD) Median (Min, Max)	42 0.19 (0.55) 0 (0, 2)	52 0.10 (0.41) 0 (0, 2)	39 0.21 (0.62) 0 (0, 3)	34 0 (0) 0 (0)	167 0.13 (0.47) 0 (0, 3)	0.21	
All (n) Mean (SD) Median (Min, Max)	81 0.26 (0.70) 0 (0, 3)	79 0.06 (0.33) 0 (0, 2)	79 0.22 (0.67) 0 (0, 4)	83 0 (0) 0 (0)	322 0.13 (0.52) 0 (0, 4)	<0.001	1v4(p=0.01); 3v4(p=0.04)

Notes: Between males and females (p=0.78); * - One way ANOVA*

Appendix BB – Fluoride concentration measured from collected drink (µg/ml) and food (µg/g) samples consumed by 4 and 8 year old study participants in the present study by area* at the time of the administration of the food frequency questionnaire (FFQ).

Type of drinks and foods∞	Area 1 (Urban, Higher F)		Area 2 (Rural, Higher F)		Area 3 (Urban, Lower F)		Area 4 (Rural, Lower F)	
	No. of samples+	F conc. (µg/ml or µg/g)	No. of samples+	F conc. (µg/ml or µg/g)	No. of samples+	F conc. (µg/ml or µg/g)	No. of samples+	F conc. (µg/ml or µg/g)
Drink groups								
DG6	5	0.139	0	-	1	0.487	0	-
DG7	2	0.091	2	0.026	1	0.013	2	0.033
DG14	0	-	0	-	2	0.07	0	-
Food groups								
FG1	12	0.902	20	1.611	23	0.757	36	1.22
FG5	11	0.274	7	6.117	11	0.454	14	0.193
FG6	12	0.118	1	0.236	12	0.123	2	0.069
FG7	6	0.279	1	1.176	8	0.256	2	0.293
FG8	56	0.345	50	1.019	47	0.427	63	0.239
FG12	2	0.404	0	-	0	-	0	-
FG15	32	0.196	42	0.437	53	0.207	59	0.316
FG17	6	0.123	5	0.05	4	0.06	2	0.137

Notes: ∞ *See Table 6.3 for drink and food group codes*

** Range of drinking water F (ppmF): Area 1: 0 – 1; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6*

Range of cooking water F (ppmF): Area 1:0 – 1; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6

+ No. of drinks and foods collected. All samples pooled and analysed as one pooled sample per drink or food group

Appendix BC – Total amount of drink (ml) and food (g) consumed by 125 four and eight year olds based on the results of the FFQ.

Drink group*	Consumption		Food group*	Consumption	
	No. of children	Total amount of drink (L) consumed		No. of children	Total amount of food (kg) consumed
DG1	22[a]	32.7[a]	FG1	121	7.6
DG2	102[a]	148.4[a]	FG2	4[b]	1.0[b]
DG3	39[a]	53.5[a]	FG3	98	25.7
DG4	95	8.2	FG4	1[b]	0.002[b]
DG5	75	4.3	FG5	123	32.5
DG6	118	16.7	FG6	125	12.6
DG7	111	13.6	FG7	115	29.9
DG8	63	0.9	FG8	125	62.0
DG9	8	1.8	FG9	1[b]	0.6[b]
DG10	7[b]	0.3[b]	FG10	125	8.1
DG11	37	2.3	FG11	19	0.7
DG12	1[b]	0.2[b]	FG12	16	2.3
DG13	42	2.1	FG13	19	2.3
DG14	121	5.8	FG14	12	0.2
			FG15	123	20.9
			FG16	0[b]	0[b]
			FG17	125	2.8
Total		**290.81**	**Total**		**209.20**

Notes: ** Check Table 6.3 for codes used for drink and food groups*
a 125 participants drank tap/borehole/sachet water (One type of water (n=95),2 types of water(n=24); 3 types of water(n=6) therefore some children were included more than once.
b Drink and foods consumed by less than 10 participants were ignored subsequently in the analysis strategy.

Appendix BD– Estimated total amount of drink (ml) and food (g) consumed daily by 4 and 8 year olds based on the results of the FFQ and source of fluoride concentration.

Drink/Food group*	Consumption		Source of information for F concentration
	No. of children	Total amount of drink (L) or food (Kg) consumed	
Drink group			
DG1-DG3	163[a]	234.6[b]	Individual collection[c]
DG4	95	8.2	PS + UK F database[d]
DG5	75	4.3	Individual collection[c]
DG6	118	16.7	Individual collection[c]
DG7	111	13.6	See Appendix BB
DG8	63	0.9	UK F. database
DG11	37	2.3	Individual collection[c]
DG13	42	2.1	Individual collection[c]
DG14	121	5.8	See Appendix BB
Food group			
FG1	121	7.6	See Appendix BB
FG3	98	25.7	UK F. database[c]
FG5	123	32.5	See Appendix BB
FG6	125	12.6	See Appendix BB
FG7	115	29.9	See Appendix BB
FG8	125	62.0	See Appendix BB
FG10	125	8.1	UK F database
FG11	19	0.7	UK F. database
FG12	16	2.3	See Appendix BB
FG13	19	2.3	UK F database
FG14	12	0.2	UK F. database
FG15	123	20.9	See Appendix BB
FG17	125	28.4	See Appendix BB

Notes: * *Check Table 6.1 for codes used for drink and food groups; PS – Present study;*
a Drink groups D1 to D3 were combined.
b Total amount of drinking water consumed by 163 participants who drank tap/borehole/sachet water
c Individual drinking water collected and F concentration analysed
d Mean F concentration of community water supply of Area 2 obtained from pilot study and UK F database

Appendix BE – Final fluoride concentration of all drinks (µg/ml) and foods (µg/g) consumed by 4 and 8 year olds based on adopted strategy by area*.

Type of drinks and foods∞	Area 1 (Urban, Higher F)		Area 2 (Rural, Higher F)		Area 3 (Urban, Lower F)		Area 4 (Rural, Lower F)	
	F conc. (µg/ml or µg/g)	Source of information for F concentration	F conc. (µg/ml or µg/g)	Source of information for F concentration	F conc. (µg/ml or µg/g)	Source of information for F concentration	F conc. (µg/ml or µg/g)	Source of information for F concentration
Drink group								
DG1-DG3	[a]	Ind. collection[a]	[a]	Ind. collection[a]	[a]	Ind. collection[a]	[a]	Ind. collection[a]
DG4	1.933	UK F database	3.607[b]	PS & UK F database[c]	0.607	UK F database	0.607	UK F database
DG5	[a]	Ind. collection[a]	[a]	Ind. collection[a]	[a]	Ind. collection[a]	[a]	Ind. collection[a]
DG6	[a]	Ind. collection[a]	[a]	Ind. collection[a]	[a]	Ind. collection[a]	[a]	Ind. collection[a]
DG7	0.091	See Appendix BB	0.026	See Appendix BB	0.013	See Appendix BB	0.033	See Appendix BB
DG8	0.132	UK F database	0.132	UK F database	0.132	UK F database	0.132	UK F database
DG11	[a]	Ind. collection[a]	[a]	Ind. collection[a]	[a]	Ind. collection[a]	[a]	Ind. collection[a]
DG13	[a]	Ind collection[a]	[a]	Ind. collection[a]	[a]	Ind. collection[a]	[a]	Ind. collection[a]
DG14	0.070	See Appendix BB	0.070	See Appendix BB	0.070	See Appendix BB	0.070	See Appendix BB
Food group								
F1	0.902	See Appendix BB	1.611	See Appendix BB	0.757	See Appendix BB	1.22	See Appendix BB
F3	0.016	UK F database	0.016	UK F database	0.016	UK F database	0.016	UK F database
F5	0.274	See Appendix BB	6.117	See Appendix BB	0.454	See Appendix BB	0.193	See Appendix BB
F6	0.118	See Appendix BB	0.236	See Appendix BB	0.123	See Appendix BB	0.069	See Appendix BB
F7	0.279	See Appendix BB	1.176	See Appendix BB	0.256	See Appendix BB	0.293	See Appendix BB
F8	0.345	See Appendix BB	1.019	See Appendix BB	0.427	See Appendix BB	0.239	See Appendix BB
F10	0.105	UK F database	0.105	UK F database	0.105	UK F database	0.105	UK F database
F11	0.877	UK F database	0.877	UK F database	0.877	UK F database	0.877	UK F database
F12	0.404	See Appendix BB	0.404	See Appendix BB	0.404	See Appendix BB	0.404	See Appendix BB
F13	0.075	UK F database	0.075	UK F database	0.075	UK F database	0.075	UK F database
F14	0.058	UK F database	0.058	UK F database	0.058	UK F database	0.058	UK F database
F15	0.196	See Appendix BB	0.437	See Appendix BB	0.207	See Appendix BB	0.316	See Appendix BB
F17	0.123	See Appendix BB	0.050	See Appendix BB	0.060	See Appendix BB	0.137	See Appendix BB

Notes: ∞*Check Table 6.3 for codes used for drink and food groups; Ind. collection – Individual collection*

** Range of drinking water F concentration (ppmF): Area 1: 0 – 1; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6*

Range of cooking water F concentration (ppmF): Area 1: 0 – 1; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6

PS – Present Study; [a] – F concentration of individual drinking water collected; b – Mean F concentration of Area 2 obtained from pilot study and UK F database

Appendix BF - Mean (SD) and median (minimum, maximum) daily dietary drink (L/day) and food (kg/day) consumption among 4 year olds by area*.

Drink and Food groups∞	Area 1 (Urban, Higher F) (n=16)		Area 2 (Rural, Higher F) (n=15)		Area 3 (Urban, Lower F) (n=17)		Area 4 (Rural, Lower F) (n=13)		All Areas (n=61)	
	Mean(SD) L/kg per day[1]	Median (Min., Max.)	Mean(SD) L/kg per day[1]	Median (Min., Max.)	Mean(SD) L/kg per day[1]	Median (Min., Max.)	Mean(SD) L/kg per day[1]	Median (Min., Max.)	Mean(SD) L/kg per day[1]	Median (Min., Max.)
Drinks										
DG1-DG3	1.9 (2.2)	1.4 (0.3, 9.9)	1.8 (0.6)	1.5 (0.6, 2.8)	1.8 (0.7)	1.9 (1.0, 3.0)	1.2 (0.4)	1.3 (0.4, 1.7)	1.7 (1.2)	1.5 (0.01, 9.9)
DG4	0.1 (0.2)	0 (0, 0.5)	0.1 (0.1)	0.04 (0, 0.3)	0.03 (0.1)	0.03 (0, 0.3)	0.05 (0.05)	0.03 (0, 0.2)	0.05 (0.1)	0.03 (0, 0.5)
DG5	0.01 (0.03)	0 (0, 0.1)	0.03 (0.1)	0 (0, 0.2)	0.01 (0.02)	0.01 (0, 0.05)	0.02 (0.03)	0.02 (0, 0.1)	0.02 (0.03)	0.002 (0, 0.2)
DG6	0.1 (0.1)	0.1 (0, 0.5)	0.2 (0.2)	0.07 (0, 0.5)	0.1 (0.1)	0.1 (0.03, 0.2)	0.1 (0.2)	0.05 (0.02, 0.7)	0.1 (0.1)	0.1 (0, 0.7)
DG7	0.01 (0.03)	0.003 (0, 0.1)	0.02 (0.04)	0 (0, 0.2)	0.02 (0.02)	0 (0, 0.2)	0.02 (0.1)	0 (0, 0.3)	0.02 (0.05)	0 (0, 0.3)
DG8	0.01 (0.01)	0.01 (0, 0.03)	0.01 (0.01)	0.01 (0, 0.03)	0.01 (0.01)	0 (0, 0.04)	0.01 (0.03)	0 (0, 0.1)	0.01 (0.01)	0 (0, 0.1)
DG11	0.01 (0.02)	0 (0, 0.1)	0.01 (0.01)	0 (0, 0.03)	0.03 (0.1)	0 (0, 0.2)	0 (0)	0 (0, 0)	0.01 (0.04)	0 (0, 0.2)
DG13	0.01 (0.02)	0 (0, 0.1)	0.01 (0.02)	0 (0, 0.1)	0.01 (0.03)	0 (0, 0.1)	0.01 (0.02)	0 (0, 0.06)	0.01 (0.02)	0 (0, 0.1)
DG14	0.03 (0.1)	0.01 (0, 0.2)	0.05 (0.04)	0.03 (0.003, 0.2)	0.1 (0.04)	0.1 (6, 0.2)	0.04 (0.03)	0.04 (0.01, 0.1)	0.04 (0.04)	0.04 (0, 0.2)
All drinks	2.1 (2.2)	1.7 (0.4, 10.2)	2.1 (0.8)	1.8 (0.7, 3.7)	2.0 (0.7)	2.0 (1.21, 3.4)	1.4 (0.5)	1.4 (0.5, 2.2)	1.9 (1.3)	1.7 (0.4, 10.2)
Foods										
FG1	0.04 (0.04)	0.03 (0, 0.1)	0.1 (0.1)	0.05 (0, 0.3)	0.1 (0.1)	0.1 (0.01, 0.1)	0.04 (0.04)	0.02 (0.01, 0.2)	0.1 (0.1)	0.03 (0, 0.3)
FG3	0.1 (0.1)	0.1 (0, 0.4)	0.2 (0.3)	0.1 (0, 1.0)	0.1 (0.1)	0.05 (0, 0.4)	0.3 (0.2)	0.2 (0, 0.4)	0.2 (0.2)	0.1 (0, 1.0)
FG5	0.3 (0.2)	0.3 (0.1, 0.7)	0.3 (0.2)	0.4 (0.01, 0.7)	0.2 (0.1)	0.1 (0, 0.2)	0.2 (0.05)	0.2 (0.1, 0.3)	0.3 (0.2)	0.3 (0, 0.7)
FG6	0.1 (0.1)	0.04 (0.01, 0.5)	0.2 (0.3)	0.04 (0.01, 1.0)	0.1 (0.1)	0.1 (0, 0.2)	0.05 (0.4)	0.04 (0.01, 0.1)	0.1 (0.2)	0.04 (0, 1.0)
FG7	0.1 (0.1)	0.2 (0, 0.4)	0.3 (0.5)	0.1 (0, 2.2)	0.3 (0.3)	0.3 (0, 1.4)	0.4 (0.4)	0.2 (0, 1.7)	0.3 (0.4)	0.2 (0, 21)
FG8	0.4 (0.1)	0.3 (0.2, 0.5)	0.4 (0.2)	0.5 (0.1, 0.6)	0.4 (0.3)	0.2 (0.1, 1.4)	0.4 (0.1)	0.5 (0.1, 0.5)	0.4 (0.2)	0.3 (0.1, 1.4)
FG10	0.04 (0.04)	0.03 (0.01, 0.1)	0.06 (0.04)	0.1 (0.01, 0.1)	0.05 (0.02)	0.1 (0.01, 0.1)	0.05 (0.02)	0.1 (0.01, 0.1)	0.05 (0.03)	0.1 (0.01, 0.1)
FG11	0.01 (0.02)	0 (0, 0.1)	0.01 (0.02)	0 (0, 0.06)	0.01 (0.02)	0 (0, 0.1)	0 (0)	0 (0, 0)	0.01 (0.02)	0 (0, 0.1)
FG12	0.04 (0.1)	0 (0, 0.3)	0 (0)	0 (0)	0.003 (0.01)	0 (0, 0.02)	0 (0)	0 (0, 0)	0.01 (0.1)	0 (0, 0.3)
FG13	0.02 (0.03)	0.01 (0, 0.1)	0.02 (0.02)	0.01 (0, 0.4)	0.02 (0.02)	0.01 (0, 0.04)	0.1 (0.1)	0.004 (0, 0.004)	0.02 (0.02)	0.1 (0, 0.1)
FG14	0.004 (0.02)	0 (0, 0.1)	0.003 (0.01)	0 (0, 0.03)	0.002 (0.01)	0 (0, 0.04)	0 (0)	0 (0, 0)	0.002 (0.01)	0 (0, 0.1)
FG15	0.06 (0.06)	0.04 (0.003, 0.2)	0.2 (0.1)	0.1 (0.04, 0.4)	0.1 (0.1)	0.1 (0, 0.4)	0.3 (0.2)	0.2 (0.03, 0.8)	0.2 (0.2)	0.1 (3, 0.8)
FG17	0.02 (0.01)	0.02 (0.01, 0.02)	0.03 (0.03)	0.03 (0.004, 0.1)	0.02 (0.01)	0.03 (0, 0.03)	0.03 (0.02)	0.03 (0.003, 0.1)	0.02 (0.02)	0.03 (0.003, 0.1)
All foods	1.2 (0.4)	1.2 (0.5, 2.1)	1.8 (1.0)	1.5 (0.9, 5.1)	1.4 (0.4)	1.3 (0.8, 2.4)	1.6 (0.4)	1.7 (1.0, 2.5)	1.5 (0.7)	1.4 (0.5, 5.1)

Notes: ∞*Check Table 6.3 for codes used for drink and food groups;* [1] *– One way ANOVA p >0.05 across areas*

** Range of drinking water F concentration (ppmF): Area 1: 0 – 1; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6*

Range of cooking water F concentration (ppmF): Area 1: 0 – 1; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6

Appendix BG – Mean(SD) daily dietary fluoride intake and mean percentage contribution to total daily dietary fluoride intake (µg/day) from drinks and foods consumed 4 year olds by area*.

Dietary sources∞	Area 1 (Urban, Higher F) (n=16)		Area 2 (Rural, Higher F) (n=15)		Area 3 (Urban, Lower F) (n=17)		Area 4 (Rural, Lower F) (n=13)		All areas (n=61)	
	Mean (SD) (µg/day)[1]	Mean (SD)%	Mean (SD) (µg/day)[1]	Mean (SD)%	Mean (SD) (µg/day)[1]	Mean (SD)%	Mean (SD) (µg/day)[1]	Mean (SD)%	Mean (SD) (µg/day)[1]	Mean (SD)%
Drink group										
DG1-DG3	167.8 (307.2)	18 (26)	1002 (1287.8)	16 (18)	26.9 (59.2)	5 (11)	122.2 (179.0)	17 (20)	324.0 (757.2)	14 (20)
DG4	123.6 (299.7)	12 (25)	233.7 (264.3)	6 (3)	20.6 (30.1)	4 (5)	28.1 (32.2)	5 (4)	101.6 (216.1)	7 (14)
DG5	0.3 (1.1)	0 (0)	6.4 (17.0)	0 (0)	0.1 (0.2)	0 (0)	0.6 (1.0)	0 (0)	1.8 (8.7)	0 (0)
DG6	11.6 (25.1)	2 (3)	118.8 (253.2)	2 (5)	1.0 (2.3)	0 (1)	12.7 (22.7)	2 (3)	35.2 (132.5)	1 (3)
DG7	1.1 (2.5)	0 (0)	0.4 (1.1)	0 (0)	0.2 (0.7)	0 (0)	0.8 (2.9)	0 (1)	0.6 (1.9)	0 (0)
DG8	1.0 (1.0)	0 (0)	0.9 (1.2)	0 (0)	0.9 (1.8)	0 (0)	1.2 (4.3)	0 (1)	1.0 (2.3)	0 (0)
DG11	0.2 (0.7)	0 (0)	2.6 (6.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.7 (3.3)	0 (0)
DG13	0 (0)	0 (0)	2.9 (6.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.7 (3.2)	0 (0)
DG14	2.4 (3.4)	1 (1)	3.2 (3.0)	0 (0)	3.8 (3.1)	1 (1)	3.1 (2.0)	1 (1)	3.1 (3.1)	1 (1)
Drinks	308.0 (74.7)	33 (30)	1371.3 (1356.0)	24 (20)	53.4 (67.5)	10 (12)	168.7 (202.4)	25 (21)	468.8 (869.9)	23 (23)
Food group										
FG1	35.9 (35.4)	7 (8)	132.3 (175.7)	3 (4)	54.0 (45.1)	11 (9)	42.7 (44.4)	9 (9)	66.1 (99.7)	8 (8)
FG3	2.3 (2.3)	1 (1)	3.5 (5.2)	0 (0)	1.7 (1.9)	0 (1)	4.2 (2.7)	1 (1)	2.8 (3.3)	1 (1)
FG5	91.9 (56.9)	19 (13)	1786.3 (1230.7)	33 (21)	76.2 (39.5)	16 (10)	42.3 (10.5)	9 (5)	493.6 (953.4)	19 (16)
FG6	8.1 (13.8)	2 (1)	310.5 (542.8)	5 (7)	10.7 (9.1)	2 (2)	3.4 (2.9)	1 (1)	82.2 (293.4)	2 (4)
FG7	39.9 (28.5)	9 (6)	359.0 (635.4)	7 (9)	75.6 (85.3)	16 (16)	102.9 (131.4)	19 (18)	141.8 (340.5)	13 (14)
FG8	105.6 (39.0)	22 (12)	433.1 (188.3)	10 (5)	177.1 (135.9)	35 (17)	85.6 (28.1)	19 (9)	201.8 (180.8)	22 (15)
FG10	4.2 (3.8)	1 (1)	6.0 (3.7)	0 (0)	5.2 (1.9)	1 (1)	5.0 (2.1)	1 (1)	5.1 (3.0)	1 (1)
FG11	4.6 (18.2)	1 (3)	4.9 (13.9)	0 (1)	5.8 (13.3)	1 (2)	0 (0)	0 (0)	4.0 (13.4)	1 (2)
FG12	17.6 (37.3)	4 (8)	0 (0)	0 (0)	1.1 (3.0)	0 (1)	0 (0)	0 (0)	4.9 (20.2)	1 (4)
FG13	1.6 (2.1)	0 (1)	1.2 (1.2)	0 (0)	1.2 (1.30	0 (0)	0.5 (0.8)	0 (0)	1.1 (1.5)	0 (0)
FG14	0.2 (0.7)	0 (0)	0.1 (0.3)	0 (0)	0.1 (0.40	0 (0)	0 (0)	0 (0)	0.1 (0.4)	0 (0)
FG15	12.2 (11.7)	2 (2)	912.2 (750.0)	19 (14)	26.1 (28.0)	6 (6)	81.0 (72.1)	15 (12)	252.0 (526.8)	10 (12)
FG17	2.5 (0.9)	1 (0)	1.4 (1.4)	0 (0)	1.3 (1.50	0 (0)	3.5 (3.3)	1 (2)	2.1 (1.9)	0 (1)
Foods	326.5 (111.8)	67 (30)	3950.6 (2111.5)	76 (20)	435.9 (137.0)	90 (12)	371.2 (129.8)	75 (21)	1257.7 (1859.5)	77 (23)
Drinks and foods	634.6 (405.5)	100	5321.8 (2332.0)	100	489.2 (145.1)	100	539.8 (231.4)	100	1726.5 (2369.5)	100

Notes: ∞ *- See Table 6.3 for codes used for drink and food groups;* [1] *– One way ANOVA p<0.01 across areas; Post-Hoc Test (1vs2 p<0.01; 2vs3p<0.01; 2vs4p<0.01)*

** Range of drinking water F concentration (ppmF): Area 1: 0 – 1; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6*

Range of cooking water F concentration (ppmF): Area 1:0 – 1; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6

Appendix BH - Median (minimum, maximum) daily dietary fluoride intake (µg/day) by drink and food groups consumed by 4 year old study participants by area*.

Dietary sources∞	Area 1 (Urban, Higher F) (n=16)		Area 2 (Rural, Higher F) (n=15)		Area 3 (Urban, Lower F) (n=17)		Area 4 (Rural, Lower F) (n=13)		All areas (n=61)	
	Median (µg/day)	(Min., Max.)	Median (µg/day)	(Min., Max.)	Median (µg/day)	(Min., Max.)	Median (µg/day)	(Min., Max.)	Median (µg/day)	(Min., Max.)
Drink group										
DG1-DG3	9.4	(0, 1015.2)	676.8	(0, 5016)	0.0	(0, 181.4)	75.2	(0, 627)	12.5	(0, 5016)
DG4	0.6	(0, 966.5)	130.9	(0, 932.2)	18.0	(0, 126.3)	18.0	(0, 126.3)	18.0	(0, 982.2)
DG5	0.0	(0, 4.5)	0.0	(0, 63.3)	0.0	(0, 0.8)	0.0	(0, 3.0)	0.0	(0, 63.3)
DG6	0.1	(0, 95.2)	20.4	(0, 952)	0.0	(0, 9.0)	2.2	(0, 71.4)	0.0	(0, 952)
DG7	0.2	(0, 9.6)	0.0	(0, 4.2)	0.0	(0, 2.7)	0.0	(0, 10.4)	0.0	(0, 10.4)
DG8	0.7	(0, 3.4)	0.7	(0, 4.3)	0.0	(0, 5.1)	1.2	(0, 15.4)	0.0	(0, 15.4)
DG11	0.0	(0, 2.8)	0.0	(0, 18.2)	0.0	(0, 0)	0.0	(0, 0)	0.0	(0, 18.2)
DG13	0.0	(0, 0.2)	0.0	(0, 18.1)	0.0	(0, 105.5)	0.0	(0, 0)	0.0	(0, 105.5)
DG14	0.6	(0, 2.3)	2.0	(0.2, 10.5)	3.5	(0.4, 10.5)	2.5	(0.8, 8.2)	35.0	(0, 175)
All drinks	74.7	(1.4, 1034.2)	1010.5	(6.8, 5134.4)	23.3	(4.4, 223.4)	111.9	(14.3, 677.7)	117.5	(1.4, 5134.4)
Food group										
FG1	22.6	(0, 112.8)	78.3	(0, 470.4)	47.39	(3.7, 110.5)	25.5	(11.8, 178.1)	31.6	(0, 470.4)
FG3	1.5	(0, 5.8)	1.5	(0, 15.4)	0.73	(0, 7.0)	3.5	(0, 7.0)	2.2	(0, 15.4)
FG5	68.0	(34, 203.9)	2397.9	(54.4, 4551)	56.30	(0, 112.6)	47.9	(23.9, 47.9)	68.0	(0, 4551)
FG6	4.1	(1, 57.1)	58.1	(13.5, 1625.5)	12.76	(0, 29.8)	2.4	(0.6, 8.6)	8.2	(0, 1625.5)
FG7	44.7	(0, 114.8)	145.1	(0, 2568.4)	63.21	(0, 368.6)	48.2	(0,5, 6.3)	48.2	(0, 2568.4)
FG8	92.0	(67.4, 157.3)	464.7	(132.8, 648.1)	97.36	(41.8, 584.1)	109.0	(31.1, 109.0)	109.0	31.1, 648.1)
FG10	3.3	(0.9, 13)	6.5	(0.9, 13)	6.51	(1.4, 6.5)	6.5	(0.9, 6.5)	6.4	(0.9, 13)
FG11	0.0	(0, 72.8)	0.0	(0, 50.9)	0.00	(0, 50.9)	0.0	(0, 0)	0.0	(0, 72.8)
FG12	0.0	(0, 127.4)	0.0	(0, 0)	0.00	(0, 9.1)	0.0	(0, 0)	0.0	(0, 127.4)
FG13	0.5	(0, 6.1)	0.9	(0, 3.1)	0.30	(0.2, 3.2)	0.3	(0.1, 3.1)	0.5	(0, 6.1)
FG14	0.0	(0, 2.6)	0.0	(0, 1.3)	0.00	(0, 1.4)	0.0	(0, 0)	0.0	(0, 2.6)
FG15	8.7	(0.7, 40.4)	51.4	(223.5, 2192.3)	15.24	(0.7, 85.3)	55.8	(10.8, 260.4)	27.9	(0.7, 2192.3)
FG17	3.1	(0.9, 3.1)	1.3	(3.6, 125)	1.50	(0.2, 1.5)	3.4	(0.5, 13.7)	1.5	(0.2, 13.70)
All foods	334.3	(137, 579)	3720.2	(1113.3, 9520.7)	415.6	(263.3, 802.1)	369.5	(189.4, 698.7)	405.2	(137, 9520.7)
All drinks and foods	481.0	(224.1,1363.1)	(4798.3)	(1120.1, 1053.1)	443.6	(298.4, 823.6)	506.7	(203.7, 960.6)	604.9	(203.7, 10531.1)

Notes: ∞ *- See Table 6.3 for codes used for drink and food groups*

** Range of drinking water F concentration (ppmF): Area 1: 0 – 0.4; Area 2: 0 – 4; Area 3: 0 – 0.2 & Area 4: 0 – 0.6*

Range of cooking water F concentration (ppmF): Area 1:0 – 0.4; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6

Appendix BI – Median (Minimum, maximum) percentage contribution to total daily dietary fluoride intake (µg/day) by drink and food groups among 4 year old study participants by area*.

Dietary sources∞	Area 1 (Urban, Higher F) (n=16)	Area 2 (Rural, Higher F) (n=15)	Area 3 (Urban, Lower F) (n=17)	Area 4 (Rural, Lower F) (n=13)	All areas (n=61)
	% contribution to total dietary fluoride intake	% contribution to total dietary fluoride intake	% contribution to total dietary fluoride intake	% contribution to total dietary fluoride intake	% contribution to total dietary fluoride intake
	Median (Min., Max.)	Median (Min., Max.)	Median (Min., Max.)	Median (Min., Max.)	Median (Min., Max.)
Drink group					
DG1-DG3	0.75 (0, 74)	18.48 (0, 75)	0 (0, 29)	10.27 (0, 65)	1.49 (0, 75)
DG4	0.17 (0, 81)	3.28 (0, 50)	2.77 (0, 21)	4.34 (0, 14)	3.12 (0, 81)
DG5	0 (0, 1)	0 (0, 2)	0 (0, 0)	0 (0, 0)	0.10 (0, 2)
DG6	0.1 (0, 11)	0.33 (0, 18)	0 (0, 2)	0.45 (0, 12)	0 (0, 18)
DG7	0.02 (0, 1)	0 (0, 0)	0 (0, 1)	0 (0, 2)	0 (0, 2)
DG8	0.12 (0, 1)	0.01 (0, 1)	0 (0, 1)	0 (0, 3)	0 (0, 3)
DG11	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
DG13	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
DG14	0.13 (0, 3)	0.05 (0, 0)	0.57 (0, 2)	0.59 (0, 3)	0.27 (0, 3)
All drinks	26.28 (0.40, 80.90)	17.04 (0.60, 71.50)	5.80 (1.04, 36.14)	16.47 (3.7, 70.50)	14.76 (0.40, 80.90)
Food group					
FG1	3.80 (0, 27)	1.60 (0, 19)	10.38 (1, 28)	5.81 (2, 35)	4.40 (0, 43)
FG3	0.32 (0, 2)	0.03 (0, 2)	0.16 (0, 2)	0.85 (0, 2)	0.16 (0, 2)
FG5	16.01 (3, 60)	42.52 (7, 79)	14.43 (0, 35)	8.40 (4, 23)	14.43 (0, 79)
FG6	1.44 (0, 5)	0.40 (0, 5)	2.18 (0, 6)	0.49 (0, 2)	0.74 (0, 6)
FG7	8.59 (0, 19)	4.33 (0, 44)	9.72 (0, 57)	11.93 (0, 62)	8.89 (0, 62)
FG8	20.13 (5, 52)	9.09 (2, 30)	32.16 (6, 71)	19.13 (6, 32)	20.19 (2, 71)
FG10	0.52 (0, 4)	0.12 (0, 1)	1.04 (0, 2)	1.07 (0, 3)	0.66 (0, 4)
FG11	0 (0, 12)	0 (0, 7)	0 (0, 8)	0 (0, 0)	0 (0, 12)
FG12	0 (0, 22)	0 (0, 0)	0 (0, 2)	0 (0, 0)	0 (0, 22)
FG13	0.10 (0, 2)	0.01(0, 0)	0.08 (0, 1)	0.06 (0, 1)	0.06 (0, 2)
FG14	0 (0, 1)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 1)
FG15	1.19 (0, 8)	1.69 (0, 7)	2.8 (0, 20)	10.66 (3, 46)	2.90 (0, 46)
FG17	0.47 (0, 1)	0.03 (0, 0)	0.31 (0, 1)	0.60 (0, 7)	0.25 (0, 7)
All foods	73.72 (19.1, 99.60)	82.96 (28.50, 99.40)	94.20 (63.90, 99)	83.53 (29.50, 96.30)	85.24 (19.10, 99.60)
All drinks and foods	100.0	100.0	100.0	100.0	100.0

Notes: ∞ *- See Table 6.3 for codes used for drink and food groups*

** Range of drinking water F concentration (ppmF): Area 1: 0 – 0.4; Area 2: 0 – 4; Area 3: 0 – 0.2 & Area 4: 0 – 0.6*

Range of cooking water F concentration (ppmF): Area 1:0 – 0.4; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6

Appendix BJ – Mean (SD) and median (minimum, maximum) daily dietary drink and food consumption (L/day) and (kg/day) among 8 year olds by area*.

Dietary sources∞	Area 1 (Urban, Higher F) (n=13)		Area 2 (Rural, Higher F) (n=14)		Area 3 (Urban, Lower F) (n=17)		Area 4 (Rural, Lower F) (n=20)		All Areas (n=64)	
	Mean(SD) L/kg per day1	Median (Min., Max.)	Mean(SD) L/kg per day1	Median (Min., Max.)	Mean(SD) L/kg per day1	Median (Min., Max.)	Mean(SD) L/kg per day1	Median (Min., Max.)	Mean(SD) L/kg per day1	Median (Min., Max.)
Drinks										
DG1-DG3	3.5 (2.6)	2.3 (0.8, 8.0)	1.8 (0.8)	1.5 (0.7, 3.0)	1.7 (1.0)	1.5 (0.4, 4.4)	1.6 (0.5)	1.5 (0.8, 2.8)	2.1 (1.5)	1.5 (0.4, 8.0)
DG4	0.1 (0.1)	0.1 (0, 0.5)	0.1 (0.2)	0.04 (0, 0.7)	0.03 (0.05)	0.03 (0, 0.2)	0.1 (0.1)	0.05 (0, 0.4)	0.1 (0.1)	0.03 (0, 0.7)
DG5	0.01 (0.01)	0 (0, 0.03)	0.04 (0.05)	0.01 (0, 0.1)	0.04 (0.1)	0.01 (0, 0.3)	0.1 (0.2)	0.02 (0, 0.8)	0.1 (0.1)	0.01 (0, 0.8)
DG6	0.1 (0.2)	0.1 (0, 0.1)	0.1 (0.1)	0.1 (0, 0.2)	0.1 (0.04)	0.03 (0.03, 0.2)	0.3 (0.5)	0.03 (0, 1.6)	0.2 (0.3)	0.05 (0, 1.6)
DG7	0.02 (0.03)	0.01 (0, 0.1)	0.01 (0.01)	0.01 (0, 0.05)	0.02 (0.1)	0 (0, 0.2)	0.002 (0.01)	0 (0, 0.02)	0.01 (0.03)	0 (0, 0.2)
DG8	0.01 (0.01)	0.1 (0.002, 0.03)	0.01 (0.01)	0.01 (0, 0.03)	0.003 (0.01)	0 (0, 0.03)	0.01 (0.01)	0 (0, 0.03)	0.01 (0.01)	0.001 (0, 0.03)
DG11	0.02 (0.1)	0.002 (0, 0.2)	0.03 (0.03)	0.03 (0, 0.1)	0.04 (0.1)	0 (0, 0.4)	0.01 (0.02)	0 (0, 0.1)	0.02 (0.1)	0 (0, 0.4)
DG13	0.02 (0.1)	0 (0, 0.2)	0.05 (0.06)	0.05 (0, 0.2)	0.02 (0.1)	0 (0, 0.2)	0.02 (0.05)	0 (0, 0.2)	0.03 (0.1)	0 (0, 0.2)
DG14	0.1 (0.1)	0.04 (0.003, 0.2)	0.06 (0.05)	0.04 (0, 0.1)	0.04 (0.04)	25 (0, 0.2)	0.05 (0.05)	0.04 (0.01, 0.2)	0.05 (0.05)	0.04 (0, 0.2)
All drinks	3.8 (2.8)	2.6 (0.9, 8.5)	2.2 (0.9)	2.3 (1.0, 3.5)	1.9 (1.1)	1.6 (1.6, 4.7)	2.2 (1.0)	1.9 (1.0, 4.8)	2.4 (1.7)	1.9 (0.6, 8.5)
Foods										
FG1	0.1 (0.1)	0.02 (0, 0.3)	0.1 (0.1)	0.05 (0.01, 0.3)	0.1 (0.05)	0.04 (0.002, 0.2)	0.1 (0.1)	0.04 (0.02, 0.3)	0.1 (0.1)	0.04 (0, 0.3)
FG3	0.1 (0.1)	0.1 (0, 0.4)	0.2 (0.1)	0.2 (0, 0.3)	0.1 (0.1)	0.05 (0, 0.3)	0.5 (0.3)	0.4 (0, 0.9)	0.2 (0.2)	0.2 (0, 0.9)
FG5	0.3 (0.3)	0.1 (0.04, 0.8)	0.3 (0.2)	0.3 (0.01, 0.1)	0.2 (0.1)	0.2 (0, 0.4)	0.3 (0.1)	0.3 (0.1, 0.5)	0.3 (0.2)	0.3 (0, 0.8)
FG6	0.1 (0.1)	0.04 (0.01, 0.5)	0.2 (0.3)	0.1 (0.01, 1.0)	0.1 (0.1)	0.1 (0.01, 0.2)	0.04 (0.04)	0.04 (0.01, 0.1)	0.1 (0.2)	0.04 (0.01, 1.0)
FG7	0.2 (0.2)	0.2 (0, 0.7)	0.3 (0.2)	0.3 (0, 0.7)	0.1 (0.1)	0.1 (0, 0.2)	0.2 (0.2)	0.2 (0, 0.6)	0.2 (0.2)	0.2 (0, 0.7)
FG8	0.7 (0.6)	0.5 (0.1, 2.1)	0.4 (0.2)	0.5 (0.1, 0.6)	0.9 (1.5)	0.6 (0, 6.4)	0.4 (0.2)	0.5 (0.1, 0.9)	0.6 (0.8)	0.5 (0, 6.3)
FG10	0.1 (0.1)	0.03 (0.004, 0.3)	0.1 (0.1)	0.05 (0.01, 0.2)	0.1 (0.04)	0.1 (0.01, 0.2)	0.1 (0.3)	0.1 (0.02, 1.2)	0.1 (0.2)	0.1 (0.004, 1.2)
FG11	0.02 (0.03)	0 (0, 0.1)	0.01 (0.02)	0 (0, 0.1)	0.005 (0.01)	0 (0, 0.1)	0 (0.002)	0 (0, 0.01)	0.01 (0.02)	0 (0, 0.1)
FG12	0.1 (0.3)	0 (0, 1.1)	0.02 (0.04)	0 (0, 0.1)	0 (0)	0 (0)	0 (0)	0 (0)	0.03 (0.1)	0 (0, 1.1)
FG13	0.03 (0.05)	0.01 (0, 0.2)	0.03 (0.05)	0.02 (0, 0.1)	0.02 (0.02)	0.003 (0, 0.1)	0.02 (0.03)	0.01 (0, 0.1)	0.02 (0.04)	0.01 (0, 0.2)
FG14	0.001 (0.003)	0 (0, 0.01)	4 (10)	0 (0, 0.03)	0 (0.002)	0 (0, 0.01)	0 (0)	0 (0)	0.001 (0.01)	0 (0, 0.03)
FG15	0.1 (0.1)	0.1 (0, 0.3)	302 (392)	0.2 (0.01, 1.5)	0.1 (0.1)	0.1 (0, 0.4)	0.2 (0.3)	0.1 (0.01, 1.0)	0.2 (0.3)	0.1 (0, 1.5)
FG17	0.03 (0.03)	0.03 (0.004, 0.1)	23 (6)	0.03 (0.01, 0.03)	0.02 (0.01)	0.03 (0.01, 0.05)	0.02 (0.01)	0.02 (0, 0.03)	0.02 (0.02)	0.03 (0, 0.1)
All foods	1.8 (1.2)	1.4 (0.6, 4.4)	1.9 (0.8)	1.8 (0.9, 3.1)	1.8 (1.6)	1.5 (0.3, 7.8)	1.8 (0.7)	1.8 (0.4, 3.5)	1.8 (1.1)	1.6 (0.3, 7.8)

Notes: ∞*Check Table 6.1 for codes used for drink and food groups;* [1] *– ANOVA p >0.05 across Areas*

** Range of drinking water F concentration (ppmF): Area 1: 0 – 1; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6*

Range of cooking water F concentration (ppmF): Area 1:0 – 1; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6

Appendix BK – Mean (SD) daily dietary fluoride intake (µg/day) by drink and food groups and mean percentage contribution to total daily dietary fluoride intake among 8 year olds by area*.

Dietary sources∞	Area 1 (Urban, Higher F) (n=13)		Area 2 (Rural, Higher F) (n=14)		Area 3 (Urban, Lower F) (n=17)		Area 4 (Rural, Lower F) (n=20)		All areas (n=64)	
	Mean (SD) (µg/day)	Mean (SD)%	Mean (SD) (µg/day)	Mean (SD)%	Mean (SD) (µg/day)	Mean (SD)%	Mean (SD) (µg/day)	Mean (SD)%	Mean (SD) (µg/day)	Mean (SD)%
Drink group										
DG1-DG3	194.0 (434.1)	12 (19)	1192.3 (1279.3)	16 (16)	103.2 (148.3)	16 (21)	198.5 (275.0)	24 (25)	389.7 (766.0)	18 (21)
DG4	111.5 (255.5)	13 (21)	422.6 (745.6)	5 (9)	20.9 (29.2)	4 (5)	61.0 (81.0)	10 (13)	139.7 (391.2)	8 (12)
DG5	0.1 (0.4)	0 (0)	36.3 (83.1)	1 (1)	4.0 (13.0)	1 (2)	7.7 (15.4)	1 (2)	11.4 (41.5)	1 (1)
DG6	8.5 (29.0)	1 (1)	107.6 (209.6)	1 (2)	4.3 (6.3)	1 (1)	12.8 (31.8)	2 (4)	30.4 (106.1)	1 (2)
DG7	2.2 (2.6)	0 (0)	0.3 (0.4)	0 (0)	0.2 (0.8)	0 (0)	0.1 (0.2)	0 (0)	0.6 (1.50	0 (0)
DG8	1.6 (1.0)	0 (0)	1.0 (1.0)	0 (0)	0.4 (1.1)	0 (0)	0.8 (1.3)	0 (0)	0.9 (1.2)	0 (0)
DG11	2.6 (8.9)	0 (0)	10.5 (14.1)	0 (0)	1.4 (5.1)	0 (1)	0 (0)	0 (0)	3.2 (8.9)	0 (0)
DG13	0.8 (2.9)	0 (0)	24.7 (48.0)	0 (1)	0.2 (0.6)	0 (0)	0 (0)	0 (0)	5.62 (24.1)	0 (0)
DG14	3.7 (3.6)	1 (1)	3.9 (3.2)	0 (0)	2.4 (2.7)	0 (1)	3.6 (3.3)	1 (1)	3.38 (3.2)	1 (1)
All drinks	325.1 (482.6)	27 (24)	1799.1 (1904.7)	24 (23)	137.1 (163.0)	21 (22)	284.4 (292.0)	37 (25)	584.9 (1118.0)	28 (24)
Food group										
FG1	53.4 (71.1)	8 (7)	100.5 (113.6)	2 (2)	45.1 (35.7)	8 (7)	83.2 (98.4)	12 (11)	70.8 (85.8)	8 (9)
FG3	2.2 (2.2)	0 (0)	2.8 (1.9)	0 (0)	1.5 (2.0)	0 (0)	7.3 (4.4)	1 (1)	3.7 (3.8)	1 (1)
FG5	78.9 (77.8)	9 (5)	1952.6 (1440.6)	32 (25)	99.9 (47.7)	16 (8)	49.1 (21.2)	9 (5)	485.0 (1021.3)	16 (15)
FG6	11.2 (15.7)	2 (3)	291.0 (489.0)	5 (7)	14.8 (9.8)	3 2)	2.6 (2.4)	0 (0)	70.7 (251.5)	2 (3)
FG7	61.1 (63.8)	10 (12)	328.2 (254.0)	7 (7)	33.8 (23.6)	6 (4)	64.7 (53.7)	10 (8)	113.4 (168.5)	8 (8)
FG8	244.8 (211.9)	33 (15)	407.3 (220.1)	7 (4)	390.2 (622.1)	41 (22)	103.2 (50.1)	18 (15)	274.7 (366.9)	25 (20)
FG10	6.1 (8.6)	1 (1)	7.1 (6.1)	0 (0)	6.7 (4.4)	1 (1)	12.3 (28.1)	2 (5)	8.4 (16.5)	1 (3)
FG11	14.5 (30.1)	2 (4)	5.3 (13.8)	0 (0)	3.9 (12.6)	0 (1)	0.4 (1.6)	0 (0)	5.2 (16.7)	1 (2)
FG12	40.1 (116.0)	3 (5)	7.4 (15.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	9.8 (53.5)	1 (2)
FG13	2.2 (3.5)	0 (0)	2.5 (3.4)	0 (0)	1.2 (1.7)	0 (0)	1.2 (2.2)	0 (1)	1.7 (2.7)	0 (0)
FG14	0 (0.1)	0 (0)	0.2 (0.4)	0 (0)	0 (0.1)	0 (0)	0 (0)	0 (0)	0.1 (0.2)	0 (0)
FG15	17.4 (16.8)	4 (3)	1607.1 (2083.9)	24 (22)	25.4 (23.6)	4 (4)	69.0 (89.1)	9 (9)	385.4 (1151.1)	10 (14)
FG17	3.8 (3.6)	1 (0)	1.2 (0.3)	0 (0)	1.3 (0.7)	0 (0)	2.2 (1.3)	0 (0)	2.0 (2.0)	0 (0)
All foods	535.6 (403.0)	73 (24)	4713.0 (2134.0)	76 (23)	623.6 (667.1)	79 (22)	395.0 (186.1)	63 (25)	1428.9 (2042.0)	72 (24)
All drinks and foods	860.7 (769.7)	100	6512.1 (2452.6)	100	760.7 (646.6)	100	679.4 (302.8)	100	2013.7 (2692.2)	100

Notes: ∞ *- Check Table 6.3 for codes used for drink and food groups;* [1] *– ANOVA p<0.01; Post-Hoc Test (1vs2 p<0.01; 2vs3p<0.01; 2vs4p<0.01)*

** Range of drinking water F concentration (ppmF): Area 1: 0 – 1; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6*

Range of cooking water F concentration (ppmF): Area 1:0 – 1; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6

Appendix BL – Median (minimum, maximum) daily dietary fluoride intake (µg/day) by drink and food groups consumed by 8 year old study participants by area*.

Dietary sources∞	Area 1 (Urban, Higher F) (n=13)		Area 2 (Rural, Higher F) (n=14)		Area 3 ((Urban, Lower F) (n=17)		Area 4 (Rural, Lower F) (n=20)		All areas (n=64)	
	Median (µg/day)	(Min., Max.)	Median (µg/day)	(Min., Max.)	Median (µg/day)	(Min., Max.)	Median (µg/day)	(Min., Max.)	Median (µg/day)	(Min., Max.)
Drink group										
DG1-DG3	8.4	(0, 1253.2)	846.0	(0, 4474.2)	18.1	(0, 438.3)	94.1	(0, 940.5)	70.7	(0, 4474.2)
DG4	23.4	(0, 934.4)	150.8	(0, 2618.0)	18.0	(0, 126.3)	31.5	(0, 252.5)	18.0	(0, 2618.7)
DG5	0.0	(0, 1.5)	5.6	(0, 318.0)	0.0	(0, 54.3)	0.3	(0, 52.8)	0.0	(0, 318)
DG6	0.0	(0, 104.8)	21.4	(0, 714.0)	0.3	(0, 20.4)	2.7	(0, 143.0)	1.9	(0, 714)
DG7	1.4	(0, 9.6)	0.3	(0, 1.2)	0.0	(0, 3.1)	0.0	(0, 7.0)	0.0	(0, 9.6(
DG8	1.5	(0.3, 3.4)	0.6	(0, 3.4)	0.0	(0, 3.9)	0.0	(0, 3.4)	0.1	(0, 3.9)
DG11	0.0	(0, 32.3)	4.6	(0, 42.4)	0.0	(0, 21.2)	0.0	(0)	0.0	(0, 42.4)
DG13	0.0	(0, 10.6)	13.1	(0, 180.8)	0.0	(0, 2.4)	0.0	(0)	0.0	(0, 180.8)
DG14	2.5	(0.2, 12.3)	3.0	(0, 10.5)	1.8	(0, 10.5)	2.9	(0.7, 12.3)	2.5	(0, 12.3)
All drinks	78.9	(7.1, 1375.1)	1231.6	(0.7, 6323.3)	62.5	(14.4, 503.7)	167.2	(5.7, 973.6)	171.5	(0.7, 6323.3)
Food group										
FG1	18.9	(0, 263.4)	72.7	(7.9, 470.4)	31.6	(1.2, 110.5)	50.9	(11.80, 178.10)	42.6	(0, 470.4)
FG3	1.5	(0, 6.7)	3.5	(0, 5.1)	0.7	(0, 5.1)	7.0	(0, 7.0)	3.5	(0, 14.1)
FG5	34.0	(9.7, 214.8)	1857.4	(54.4, 4551.0)	112.6	(0, 178.0)	47.9	(23.90, 47.90)	69.9	(0, 4551.0)
FG6	4.1	(1.3, 57.1)	116.0	(14.4, 1625.5)	12.8	(1.4, 29.8)	2.4	(0.60, 8.60)	9.0	(0.8, 1625.5)
FG7	45.9	(0, 203.1)	291.7	(0, 856.1)	21.1	(0, 63.2)	48.2	(0. 506.30)	48.2	(0, 856.1)
FG8	157.3	(45, 738)	464.7	(99.6, 648.1)	271.6	(0, 2717.0)	109.0	(31.10, 109.0)	177.8	(0, 2717.0)
FG10	3.3	(0.5, 32.6)	4.9	(1.4, 19.5)	0.0	(0, 19.5)	6.5	(0.90, 6.50)	6.5	(0, 130.3)
FG11	0.0	(0, 101.7)	0.0	(0, 50.9)	0.0	(0, 50.9)	0.0	(0)	0.0	(0, (101.7)
FG12	0.0	(0, 423.0)	0.0	(0, 44.3)	0.0	(0)	0.0	(0)	0.0	(0, 423.0)
FG13	0.9	(0, 12.3)	0.0	(0, 13.1)	0.0	(0, 6.1)	0.4	(0.10, 3.10)	0.6	(0, 13.1)
FG14	0.0	(0, 0.4)	0.0	(0, 1.3)	0.0	(0, 0.3)	0.0	(0)	0.0	(0, 1.3)
FG15	11.5	(0, 60.6)	1037.3	(72.9, 8220.9)	0.0	(0, 85.3)	32.6	(10.8, 260.40)	26.9	(0, 8220.9)
FG17	3.1	(0.4, 15.4)	1.3	(0.2, 1.3)	0.2	(0, 3.0)	2.5	(0.50, 13.70)	1.5	(0, 15.4)
All foods	416.0	(168.4, 1437.5)	4380.0	(1543.0, 9653.1)	465.7	(80.0, 3109.1)	361.6	(106.2, 932.6)	477.8	(80.0, 9653.1)
All drinks and foods	423.1	(234.1, 2565.0)	6220.3	(1557.0, 10759.8)	602.9	(249.1, 3128.9)	660.5	(260.5, 1400.9)	726.6	(234.1, 10759.8)

Notes: ∞ *- Check Table 6.3 for codes used for drink and food groups;* [1] *– ANOVA p<0.01; Post-Hoc Test (1vs2 p<0.01; 2vs3p<0.01; 2vs4p<0.01)*

** Range of drinking water F concentration (ppmF): Area 1: 0 – 1; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6*

Range of cooking water F concentration (ppmF): Area 1:0 – 1; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6

Appendix BM – Median (Minimum, maximum) percentage contribution to total daily dietary fluoride intake (µg/day) by drink and food groups among 8 year old study participants by area*.

Drink and food groups∞	Area 1 (Urban, Higher F) (n=13)	Area 2 (Rural, Higher F) (n=14)	Area 3 (Urban, Lower F) (n=17)	Area 4 (Rural, Lower F) (n=20)	All areas (n=64)
	% contribution to total dietary fluoride intake	% contribution to total dietary fluoride intake	% contribution to total dietary fluoride intake	% contribution to total dietary fluoride intake	% contribution to total dietary fluoride intake
	Median (Min., Max.)	Median (Min., Max.)	Median (Min., Max.)	Median (Min., Max.)	Median (Min., Max.))
Drink group					
DG1-DG3	3.57 (0, 61)	15.29 (0, 58)	4.22 (0, 60)	18.31 (0, 69)	7.09 (0, 69)
DG4	4.28 (0, 61)	2.29 (0, 31)	2.63 (0, 22)	5.95 (0, 52)	3.54 (0, 61)
DG5	0 (0)	0.12 (0, 4)	0 (0, 6)	0.05 (0, 5)	0 (0, 6)
DG6	0 (0, 5)	0.29 (0, 8)	0.01 (0, 2)	0.47 (0, 16)	0.22 (0, 16)
DG7	0.36 (0, 1)	0 (0)	0 (0, 1)	0 (0)	0 (0, 1)
DG8	0.23 (0, 1)	0.01(0, 1)	0 (0, 1)	0 (0, 1)	0 (0, 1)
DG11	0 (0, 1)	0.04 (0, 1)	0 (0, 3)	0 (0)	0 (0, 3)
D13	0 (0, 1)	0.15 (0, 2)	0 (0, 1)	0 (0)	0 (0, 2)
DG14	0.54 (0, 2)	0.05 (0, 1)	0.29 (0, 2)	0.39 (0, 4)	0.27 (0, 4)
All drinks	15.14 (1, 67)	21.74 (0,75)	10.37 (1, 68)	35.72 (1,75)	20.85 (0, 75)
Food group					
FG1	6.14 (0, 23)	1.35 (0, 7)	4.69 (0, 24)	9.12 (2, 43)	4.93 (0, 43)
FG3	0.26 (0, 2)	0.05 (0)	0.11 (0, 1)	1.34 (0, 3)	0.15 (0, 3)
FG5	8.90 (2, 19)	26.13 (4, 88)	16.45 (0, 28)	8.34 (3, 17)	11.30 (0, 88)
FG6	0.62 (0, 10)	1.82 (0, 20)	2.19 (0, 7)	0.30 (0, 1)	0.98 (0, 20)
FG7	4.90 (0, 31)	3.29 (0, 22)	4.87 (0, 11)	8.22 (0, 30)	5.94 (0, 3)
FG8	28.82 (11, 67)	7.56 (1, 15)	40.35 (0, 87)	12.87 (6, 55)	18.86 (0, 87)
FG10	0.66 (0, 2)	0.07 (0, 1)	0.97 (0, 3)	0.82 (4, 64)	0.64 (0, 22)
F11	0 (0, 16)	0 (0, 1)	0 (0, 5)	0 (0, 1)	0 (0, 16)
1G2	0 (0, 17)	0 (0, 1)	0 (0)	0 (0)	0 (0, 17)
FG13	0.20 (0, 1)	0.02 (0)	0.05 (0, 1)	0.06 (0, 3)	0.05 (0, 3)
FG14	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
FG15	2.98 (0, 10)	21.90 (1, 76)	3.44 (0, 13)	6.35 (1, 35)	4.24 (0, 76)
FG17	0.77 (0, 1)	0.02 (0)	0.22 (0, 1)	0.29 (0, 1)	0.22 (0, 1)
All foods	84.86 (33.99)	78.26 (25, 100)	89.63 (32, 99)	64.28 (25, 99)	79.15 (25, 100)
All drinks and foods	100	100	100	100	100

Notes: ∞ *- Check Table 6.3 for codes used for drink and food groups*

** Range of drinking water F concentration (ppmF): Area 1: 0 – 0.4; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6*

Range of drinking water F concentration (ppmF): Area 1: 0 – 0.4; Area 2: 0 – 4; Area 3: 0 – 2 & Area 4: 0 – 0.6

Appendix BN - Predictive model.

Phase 1 (n=624)																			
Response or Dependent variables	**Explanatory or Independent variables (Predictors)**																		
	Age (4 & 8 yrs)	**Gender (M & F)**	**FC. DW**	**F C. CW**	**E.B .F**	**Age B. feeding stopped**	**Childhood disease**	**Tooth cleaning**	**Age teeth cleaning**	**Frequency of teeth cleaning**	**Amount of toothpaste used**	**Toothpaste exposure**	**Normal birth**	**Family history of discolored teeth**	**TDFI**	**TDFR**	**Stunting**	**Wasting**	**SNP A, C & AC**
DDE (Yes/No)	xx	xx	xx	xx	xx	xx	xx	Xx	xx	xx	xx	xx	xx	xx					
Dental fluorosis (Yes/No)	xx	xx	xx	xx	xx	xx	xx	Xx	xx	xx	xx	xx	xx	xx					
Phase 2 (n=125)																			
DDE (Yes/No)	xx	xx	xx	xx	xx	xx	xx	Xx	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx	
Dental fluorosis (Yes/No)	xx	xx	xx	xx	xx	xx	xx	Xx	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx	
Phase 3 (n=70)																			
DDE (Yes/No)	xx	xx	xx	xx	xx	xx	xx	Xx	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx
Dental fluorosis (Yes/No)	xx	xx	xx	xx	xx	xx	xx	Xx	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx

<u>Notes:</u> FC. – Fluoride concentration; DW – Drinking water; CW – Cooking water; E.B.F – Exclusive breastfeeding; B. – Breastfeeding; TDFI – Total daily fluoride intake; TDFR – Total daily fluoride retention; SNP – Single Nucleotide Polymorphism

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