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Assessment of Fluoride Intake in Endemic Areas of Ethiopia and Potential Use of Calcium Rich Foods in Mitigating Ingested Fluoride

PhD Dissertation in Food Science and Nutrition

By

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ABSTRACT

Excessive intake of fluoride is accompanied by a characteristic sequence of changes in teeth, bone and periarticular tissues. Skeletal changes lead to a variable degree of locomotor disability, ranging from simple mechanical back pain to severe, crippling and neurological impairment. Fluorosis is an important clinical and public health problem in several parts of the world. Reports indicate that 14 million Ethiopians dwelling in Ethiopian Rift Valley (ERV) are at risk of fluorosis.

In Ethiopia, there are several studies reporting fluoride content of water sources while that of dietary intake of fluoride is scarce. For the purpose of prevention and mitigatory measures, studies of all potential fluoride sources are important. The knowledge and practices of endemic communities on etiology of fluorosis may also help in its mitigation and prevention. The objective of this study is therefore to identify potential fluoride sources, fluorosis risks and to determine the level of dietary fluoride intake and its effects to suggest mitigatory means of ingested fluoride based on nutritional intervention. Moreover the assessment of the knowledge, attitude and practices of endemic community on fluoride contamination, fluorosis and prevention practices is used to devise coordinated and targeted prevention mechanisms.

Reconnaissance survey was conducted to identify three water sources having fluoride content in the range of 5-7mg/L (cause skeletal fluorosis) from three different dietary areas in Ethiopian Rift Valley. In the assessment 48 water sources were randomly selected and assessed for the level of fluoride. Out of those screened Benti in Fentale, Halaku in Adamitulu and Kobochobare in Alaba were selected to study fluoride intake and the effect of nutrient intake on fluorosis. The selection of the water source was based on service year (>10yrs), fluoride level ($5 \leq X \leq 7$ mg/L) and dietary practice of the community's using the selected water source. Focus group discussions (FGD) were conducted in each selected dietary areas to collect knowledge, attitude and practices (KAP) of the endemic community on fluoride intake and fluorosis.

Index children and their biological mothers were recruited from the three communities. The eligibility criteria for household selection were dwelling for more than 10 years, use of selected water source and availability of school age child in the household. All eligible households who are volunteered to participate in the study were recruited and informed

consent obtained (N=220). Dietary fluoride, nutrient intake and fluorosis rate of school age children (10-15 years) and biological mothers (reproductive age: 20-45 years) were assessed in the three selected fluorosis endemic areas. Duplicate plate method was used to collect foods consumed by children and mothers over 24 hours from 20 households (randomly selected) in each community and analyzed for fluoride and nutrient content. Dental fluorosis rate was determined using Dean Index. The methods of Susheela and Bhatnagar (2002) were used to assess skeletal and non-skeletal fluorosis. Household questionnaire was also used to collect socioeconomic data from the selected households. To investigate the role of nutrient supplementation in mitigation of ingested fluoride, animal experiment and human trial was conducted using calcium citrate and moringa (Moringa stenopetala) dry leaf. The effect of tea leaf on fluoride content was studied using fluoridated and non-fluoridated water.

Ninety three percent of the water (N=48) sources contained fluoride above WHO guideline value (1.5mg/L) for drinking water. Staple foods and food ingredients had relatively high fluoride content and may contribute to the total body fluoride burden. The fluoride content of most of prepared foods (1.3-3.2mg/kg) is because of fluoride contaminated water used for preparation except tea leaf (4.2-7.1mg/L) which accumulates significant amount of the chemical from soil. As a result the daily dietary fluoride intake was above tolerable daily intake (TDI). All the dietary sources; i.e., water, food and beverage contribute significant amount to the daily fluoride burden. On the other hand dietary intake of nutrients such as calcium was below recommended daily allowance. In our assessment it is observed that urinary fluoride in Fentale and Adamitulu is very high (>5mg/L) compared to Alaba. Severe and moderate dental fluorosis (≥3) was found in Alaba (20%) and Adamitulu (50%). Although drinking water fluoride level was in the range that causes skeletal fluorosis in all study areas, dental fluorosis ≥3 in Fentale (0%) is lower than the two water sources. Milk consumption was high in Fentale (100%) and low in Alaba (41%) and Adamitulu (22.3%). Rain water harvesting is common in Alaba (25%) and none in Adamitulu. The investigation showed that some stiffness of joints, tingling sensation in the extremities, stiffness in the neck movement and muscle weakness exists in all the study areas.

The result from FGD indicated that health consequences of fluoride contaminated water are fairly understood. None of the discussants mentioned the word fluoride. The knowledge and perception of the community on ingestion of fluoride was poor. Health extension workers

(HEWs) did not teach about fluoride and related health consequences. Dental fluorosis is not commonly perceived as a major problem. Adolescents feel that they were singled out in non-endemic areas. Older people have a skeletal fluorosis which interferes with their day to day activities. In those severely affected individuals, the teeth were weak and fragile creating difficulty in chewing hard foods. The consumption of vegetables and fruits is almost none. People prefer rain water than from borehole because of inconvenient taste of the later.

It can be conclude that consumption of tea, foods prepared with fluoride contaminated water and foods which have high water absorption capacity like rice might contribute to fluorosis. The daily fluoride intake of the studied communities is far above the tolerable daily intake and the subjects are under severe risk of fluorosis. However, the health consequences of fluoride contaminated water are fairly understood by the studied communities yet the emphasis given to fluorosis by health workers is very poor. In addition to the use of defluoridation technologies, dietary practice of calcium rich foods such as dairy products, finger millet, teff, vegetables and trend of harvesting and using rain water for household consumption and preparation of food might help in reducing risk of fluorosis. The use of moringa for mitigation of ingested fluoride has additional benefit of increasing the dietary intake of vitamins and minerals.

The effect of fluoride contamination and mitigatory means should get sufficient attention by the community, health workers and concerned governmental bodies. Fluorosis mitigation and prevention should be part of the work of health extension workers in the Main Ethiopian rift valley. Moreover information communication works among community as well as health promoters including health extension works should be given more emphasis in future mitigation works. Future studies should focus on intervention on defluoridation and mitigatory techniques.

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Dedication

The work is dedicated to my parents,

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and

Kebede Arfassa,

Whom I lost during the study.

ACRONYMS AND ABBREVIATIONS

AAP: American Academy of Pediatrics

AAU: Addis Ababa University

AI: Adequate Intake

ATSDR: Agency for Toxic Substances and Disease Registry

BMD: Bone Mineral Density

CDA: Canadian Dental Association

CDC: Centers for Disease Control and Prevention

CEN: European Committee for Standardization

CRM: Certified Reference Materials

CSB: Corn-Soya Blend

DHS: Demographic and Health Survey

DI: Daily Intake

DRI: Dietary Reference Intakes

EAR: Estimated Average Requirement

EFSA: European Food Safety Authority

EHE: Estimated Human Exposure

EPHI: Ethiopian Public Health Institute

ERV: Ethiopian Rift Valley

ES: Ethiopian Standard

EQSA: Ethiopian Quality and Standards Authority

EU: European Union

FAO: Food and Agricultural Organization

IAEA: International atomic Energy Agency

FDRE: Federal Democratic Republic of Ethiopia

FFQ: Food Frequency Questionnaire

FGD: Focus Group Discussions

FNB: Food and Nutrition Board

GV: Guideline Value

HDDS: Household Dietary Diversity Score

HEWs: Health extension workers

HQ: Hazard Quotient

IDDS: Individual Dietary Diversity Score

ISE: Ion-Selective Electrode

IPCS: International Program on Chemical Safety

KAP: knowledge, attitude and practice

LOAEL: least observable adverse effect

MOE: Margin of Exposure

MoWR: Ministry of Water Resources

MoWE: Ministry of Water and Energy

NFMPO: National Fluorosis Mitigation Project Office

NOAEL: No Observable Adverse Effect

NRC: National Research Council

OSC: Osteoporosis society of Canada

NDA: Panel on Dietetic Products, Nutrition and Allergies

POD: Point of Departure

RDA: Recommended Dietary Allowances

RECC: Research and Ethical Clearance Committee

RfD: Reference Dose

RiPPLE: Research-inspired Policy and Practice Learning in Ethiopia

SCCNFP: Scientific Committee on Cosmetic Product and Non-Food Products

SCF: Scientific Committee on Food

SCHER: Scientific Committee on Health and Environmental Risks

SCSEDRI: Standing Committee on Scientific Evaluation of Dietary Reference Intakes

SF: Safety Factor

SNNPR: South Nations Nationalities' People Region

SOD: Superoxide dismutase

TFI: Thylstrup-Fejerskov Index

TISAB: Total Ionic Strength Adjustment Buffer

TDI: Tolerable Daily Intake

TSIF: Tooth Surface Index of Fluorosis

FDA: Food and Drug Administration

UFs: Uncertainty Factors

UK: United Kingdom

DoH: Department of Health

UL: Upper Tolerable Intake Levels

US DHHS: Department of Health and Human Services

EEPA: Ethiopian Environmental Protection Agency

EPA: Environmental Protection Agency

WHO: World Health Organization

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CHAPTER ONE: INTRODUCTION

Fluorine ranks thirteen among the elements in order of abundance in the earth's crust. It is an inevitable part of the biosphere and human life. Fluorine combines directly with most elements and indirectly with few to form fluorides (Waldbott, 1963). Fluorides are ubiquitous in nature and are present in rocks, soil, water, plants, foods and air (Fawell *et al.*, 2006) as fluorspar (CaF_2), cryolite ($3NaF.AlF_3$) or apatite ($3Ca_3(PO_4).2Ca(F,OH,Cl)_2$ (WHO, 1994). Availability of fluoride from soil depends on the solubility of the fluoride compound, the acidity of the soil and the presence of water (WHO, 2011).

Fluoride enters human body mainly through water and food. Studies show that water is epidemiologically the most important source of dietary fluoride (75–90% of daily intake) in most areas (Kloos & Tekle Haimanot, 1999). Others indicate that considerable exposure risk is also associated with the consumption of fish bones, canned meat, vegetables, grains, local salt (magadi), drinks (especially tea) and air (WHO 1984; Malde *et al.*, 1997). In some African and Asian communities, intake of fluoride from food has been found to be higher than from water (Malde *et al.*, 2004).

Optimum fluoride intake plays an essential role in the prevention of caries while fluoride consumption above the guideline level interferes with the normal formation of tooth enamel and bones (Dean 1934 , Erdal and Buchanan, 2005) and may increase risk of dental and skeletal fluorosis (WHO, 2006; Rango *et al.*, 2012). The major sources of fluoride intake include water, beverages and foods prepared with fluoride contaminated water (Kaseva, 2006; Mandinic *et al.*, 2009; Martinez-Mier *et al.*, 2003; Malde *et al.*, 2003). Endemic fluorosis has been described in many parts of the world (WHO, 1970), including Ethiopia (Assefa *et al* 2004; Tekle-Haimanot *et al* 1990). Several studies in African countries have found a high prevalence of dental fluorosis even among populations that consume drinking water with relatively low fluoride content (El-Nadeef and Honkala, 1998; Ibrahim *et al.*, 1995; Helderman *et al.*, 1997; Tekle-Haimanot R, 1990).

The guideline value of 1.5 mg/L set by WHO (1984, 2008) for drinking water has been considered by some researchers to be too high. Critics contend that the high fluid intake in hot tropical areas increases exposure risk, as evidenced by studies showing the development of dental fluorosis from the consumption of water with fluoride concentrations as low as 0.8 mg/l (Brouwer *et al.*, 1988). Drinking water containing optimum levels of fluoride, 0.5–1.5 mg/l, depending on climatic conditions and the relative contribution of non-aqueous sources of fluoride to overall fluoride load in individuals, has been believed to confer protection against dental caries without causing fluorosis. The formula developed by Galagan and Vermillion (1957) to determine optimum fluoride levels in drinking water considers annual mean maximum temperature because water consumption is greater in hot climates. However, the inability to adequately reduce fluoride levels in most high-fluoride areas and other factors in fluorosis etiology has rendered these and most other fluoride standards unobtainable (Schuiling, 1990).

In humans, fluorosis is most easily detected in the teeth, in the form of mottling of tooth enamel known as dental fluorosis (Dean, 1934; Fejerskov *et al.*, 1996). Drinking water concentration of fluoride at 3 - 6 mg/l may cause skeletal fluorosis and at concentrations higher than 6 mg/l crippling skeletal fluorosis (WHO, 1984; Brouwer *et al.*, 1988,). Skeletal fluorosis is directly related to the level and duration of fluoride exposure (Saraux *et al.*, 1994). A study conducted in India reported neurological disorders and impaired development of intelligence due to excess fluoride (Susheela & Bhatnagar, 2002). The public health and economic importance of fluorosis is significant in many endemic areas in view of the occurrence of debilitating skeletal fluorosis in humans as well in livestock (Dunipace *et al.*, 1995). No economic impact studies of fluorosis have been conducted, but the large number of affected persons and disability of workers with crippling disease and early retirement indicate the economic impact of fluorosis (Kloos & Tekle Haimanot, 1999).

Fluoride intake has usually been calculated indirectly, based on the records of food and drinks consumed each day and to the known fluoride content of the most frequently consumed types of food and beverages (Levy *et al.*, 2010; Martínez-Mier *et al* 2009). The method of indirect calculation of fluoride intake based on diets and table values of fluoride content in the

essential components of a diet became unsatisfactory and it is being replaced by duplicate plate method (Villa et al 1999). This method consists of collecting equivalent amount of food and beverages received by a subject during the day and then analyzing the resulting homogenate to determine the fluoride content (Oganessian *et al.*, 2011; Malde *et al.*, 2001; Erdal and Buchaman 2005).

Fluoride is not generally considered an essential mineral element because humans do not require it for growth or to sustain life although its role in the prevention of dental caries (tooth decay) is well established (Nielsen, 1999). 'After oral uptake, water-soluble fluorides are rapidly and almost completely absorbed from the gastrointestinal tract, with a plasma peak level occurring after 30 minutes in contrast to the low soluble fluoride compounds (EFSA, 2006; Cerklewski, 1997; Spencer *et al*, 1981). Fluoride is absorbed in the stomach and small intestine. Approximately, 75-90 % of the fluoride ingested each day is absorbed from the alimentary tract, with higher proportions from liquids than from solids (EFSA, 2006). Absorption from the stomach occurs readily and is inversely related to the pH of the gastric contents, and most of the remaining fluoride that enters the intestine will be rapidly absorbed.

Absorbed fluoride is rapidly distributed throughout the body, where it is incorporated into calcified tissue (teeth and bones) due to its high affinity for calcium, with virtually no storage in soft tissues (WHO, 2011). There is variability in the bioavailability of fluoride from different foods. High concentrations of dietary calcium and other cations that form insoluble complexes with fluoride can reduce fluoride absorption from the gastrointestinal tract (WHO 1994). Most of fluoride is absorbed as undissociated hydrogen fluoride and absorption occurs by passive diffusion in both the stomach and the small intestine. Higher acidity of the stomach increases absorption (Ekstrand and Ehrnebo, 1979; Shulman and Vallejo, 1990). (He *et al.*, 1998)

The predominant mineral elements in bone are crystals of calcium and phosphate, known as hydroxyapatite crystals. Fluoride's high chemical reactivity and small radius allow it to either displace the larger hydroxyl (-OH) ion in the hydroxyapatite crystal, forming fluoroapatite, or

to increase crystal density by entering spaces within the hydroxyapatite crystal. Fluoroapatite is like hydroxyapatite, except that the fluoride anion substitutes for the hydroxyl anion. It has a more ordered structure and is less soluble than hydroxyapatite (Brody, 1999). As a result it hardens tooth enamel and stabilizes bone mineral (Cerklewski, 1997a).

Ninety-nine percent of the total fluoride content of the body is concentrated in calcified tissue. Body fluid and soft tissue fluoride concentrations are not under homeostatic control and reflect the recent intake (Ekstrand, 1977a, b). In bone the substitution of fluoride for hydroxyl groups in apatite forms fluoroapatite altering the mineral structure of the bone. Fluoroapatite is more regular and less acid soluble than apatite compound (Featherstone *et al*, 1983; Okazaki *et al*, 1985). Bone density increases with increasing fluoride content of bone as a consequence of an increasing fluoride intake both in animals and in humans (Turner *et al* 1992; Chachra *et al.*, 1999). This increase in bone fluoride content is accompanied by an increase in bone strength up to a certain level; after which the bone strength starts decreasing (Turner *et al.*, 1995; Chachra *et al.*, 1999). Fluoride is distributed to all tissues via the plasma (Whitford, 1996).

The presence of excessive quantities of fluoride in drinking water and food is accompanied by a characteristic sequence of changes in teeth, bone and periarticular tissues. These changes lead to variable degree of locomotors disability, ranging from simple mechanical back pain to severe and crippling locomotors and neurological impairment known as fluorosis (Chakma and Rao, 2004). Fluorosis is an important clinical and public health problem in several parts of the world. The global prevalence of fluorosis has been reported to be about 32% (Mella *et al.*, 1994). The maximum impact is felt in communities engaged in physically strenuous activities, either agricultural or industrial (Mella *et al.*, 1994; Susheela and Ghosh, 1990). The severity of fluorosis depends on when and for how long the overexposure to fluoride occurs, the individual response, weight, degree of physical activity, nutritional factors and bone growth, suggesting that similar dose of fluoride may lead to different levels of fluorosis. Other factors that may increase the individual susceptibility to dental fluorosis are altitude and renal insufficiency (Alvarez *et al.*, 2009; Akosu and Zoakah, 2008)

There are more than 23 developed and developing nations (Figure 1) that are endemic for fluorosis of which Ethiopia is among the most affected ones (Chakma and Rao, 2004; Tekele-Haimanot *et al.*, 1987). Ethiopia is of particular interest due to the predominance of extensive volcanic basalt flows (Fawell *et al.*, 2006) in the lowland and highland regions that originated from complex tectonic events. Moreover the low socio-economic level and malnutrition might exacerbate the problem (DHS 2011). Both skeletal and dental fluorosis is widely distributed in Ethiopian Rift Valley (Kloos & Tekle-Haimanot, 1999).

Figure 1: Areas of the world with high water fluoride concentrations and endemic fluorosis

Reports indicate that more than 14 million Ethiopians may be potentially at risk. A recent assessment of fluoride, fluorosis and defluoridation issues reported that, out of those at risk, approximately 85% may have already been exposed to high fluoride contamination (The Daily Monitor, 2008). In the Ethiopian Rift Valley, 41% of the drinking water sources have a fluoride concentration exceeding the WHO guideline value of 1.5 mg F^-/L (Tekle-Haimanot *et al.*, 2006) and excessive fluoride is the most serious water sanitation problem, mainly in the Ethiopian Rift Valley system affecting areas in East (Afar), Central (Oromia), the south-west (SNNPR including some parts of Gambela) as well as a few high land areas of the country. Analysis of hydro chemical, economic and demographic factors in the spatial distribution of high-fluoride domestic water sources indicates that fluorosis problem has become more serious in the Rift Valley in recent decades (Kloos & Tekle-Haimanot, 1999). On the other hand, in view of the increased emphasis on safety of drinking water, public health and water

managers give less emphasis to fluorosis in the presence of other highly prevalent life-threatening health problems (Melaku and Ismail, 2002). WHO (2008) recommends a guideline value of 1.5mg F/Lin natural fluoridated drinking water. However, where intakes are likely to exceed 6 mg/day, as in the cases in Ethiopian Rift Valley it is appropriate to consider a local guideline fluoride concentration lower than 1.5 mg/L (WHO 2008).

Fluorosis has no treatment but can be prevented through appropriate intervention if the disease is diagnosed at an early stage. Studies show that fluoride poisoning can be prevented or minimized by using alternate water sources, removing excess fluoride (defluoridation) from drinking water and by supplementation of nutrients that can bind fluoride for excretion as well anti-oxidants to reduce the toxic effect (RiPPLE, 2008; Gupta *et al.*, 1994; Susheela & Bhatnagar, 2002). Data obtained from dietary supplementation studies suggested that inadequate levels of ascorbic acid and calcium are related to the manifestation and severity of fluorosis (Srirangareddy and Srikantia 1971). According to Susheela and Bhatnagar (2002) toxic effects of fluoride can be reversed by withdrawing the fluoride source and by providing a diet adequate in protein, calcium and anti-oxidants (vitamins A, C, E and D). Several researchers mentioned that antioxidants play a protective role in fluorosis (Sridharan *et al.*, 1999; Chinoy 1978). Superoxide dismutase (SOD) and β-carotene were also recommended in effectively mitigating impaired growth due to fluoride toxicity in rats (Wang *et al.*, 1994). Calcium forms insoluble complexes with fluoride and assumed to reduce fluoride bioavailability through minimizing absorption when supplemented or calcium rich food consumed (Cerklewsk 1997b; WHO, 1994). The knowledge, attitude and practices of the community in mitigation of the problem will also helps in the mitigatory processes in several aspects (Melaku and Ismail, 2002).

In Ethiopia, the studies conducted so far mainly focused on water fluoride distribution in the country (Reimann *et al.*, 2003; Tekle-Haimanot *et al.*, 2006). Data on fluorosis status have only been conducted in limited areas (Assefa *et al.*, 2004; Wondwosen *et al*, 2004). There is very limited information on dietary fluoride intake on different age groups (Malde *et al.*, 1997; 2003; 2004; Desalegne and Zewge, 2013). The study conducted on fluoride intake of children (6-59 months) from food and beverages indicated that fluoride intake is far above the

maximum permissible level (Malde *et al.*, 2003), and that food may be a major fluoride source (Malde *et al.*, 2004). Recent study conducted on fluoride intake from locally brewed alcoholic beverages indicated accelerated development of skeletal fluorosis (Tekle-Haimanot and Haile, 2014). Defluoridation technologies have been implemented in pocket areas although the techniques did not remove fluoride as much as intended. Moreover the running costs, perceptions and lack of knowledge on fluorosis did not allow the people to fully utilize the techniques. Nutritional intervention is a new approach to mitigation of ingested fluoride and to reduce the detrimental effect of fluorosis. Locally available, calcium and anti-oxidant rich foods such as moringa leaf (Babu 2000) might help in mitigating ingested fluoride. Fresh leaf of moringa is commonly consumed as vegetable among South Omo nations.

The aim of this study is therefore

- To investigate the fluoride level of selected staple diets in the Ethiopian Rift Valley.
- To assess the knowledge, attitude and perception (KAP) of the community about fluorosis, related health problems, techniques and measures taken to tackle fluoride contamination and possible solutions to mitigate ingested fluoride.
- To determine dietary fluoride intake and associated fluorosis rate among school age children and women of reproductive age in three dietary areas in Ethiopian rift valley.
- To assess the possibility of supplementation of locally available or adaptable calcium and antioxidant rich food / food item in the mitigation of ingested fluoride.

The hypothesis in the experiment is that when calcium combines with fluoride it forms insoluble calcium fluoride in the gut and thereby reducing the bioavailability of fluoride in plasma and consequently in urine (Buzalaf and Whitford, 2011).

CHAPTER TWO: STATEMENT OF THE PROBLEM & OBJECTIVE OF THE STUDY

2.1 Statement of the problem

In the Ethiopian Rift Valley, 41% of the drinking water sources have a fluoride concentration exceeding the WHO guideline value of 1.5 mg F/L (Tekle-Haimanot *et al.*, 2006) and excessive fluoride is the most serious water sanitation problem affecting areas in Afar, Oromia and SNNPR. More than 14 million Ethiopians may be potentially at risk of fluorosis and over 10 millions may have already been exposed to high fluoride contamination (The Daily Monitor, 2008). Analysis of hydrochemical, economic and demographic factors in the spatial distribution of high-fluoride domestic water sources indicates that fluorosis problem has become more serious in the Rift Valley (Kloos & Tekle-Haimanot, 1999). Dental as well as skeletal fluorosis is endemic in the region (Kloos & Tekle-Haimanot, 1999; Tekle-Haimanot *et al.*, 1987) because of high fluoride level in the ground water that originates from extensive volcanic basalt flows (Fawell *et al.*, 2006). Moreover the low socio-economic level, recurring famine and malnutrition might exacerbate the problem (DHS 2011).

According to report of FDRE, Ministry of Water Resources (MoWR, 2001), the areas of Ethiopian Rift valley are among most affected due to scarcity of fresh surface water and the inhabitants are entirely dependent up on ground water (boreholes). In addition, the area is among dry climatic zone; with low elevation and high annual mean temperature, the daily intake of water is expected to be high. Hence, the population is certainly exposed to risk of fluorosis. On the other hand, in view of the increased emphasis on safety of drinking water, public health and water managers give less emphasis to fluorosis in the presence of other highly prevalent, acute and life-threatening health problems (Melaku and Ismail, 2002).

In view of aforementioned grave problems of fluoride ingestion and its risks it is necessary to

- Conduct dietary fluoride intake assessment to identify magnitude of exposure and fluorosis risk factors.

- Investigate endemic community KAP on fluorosis risks and mitigation
- Document and characterize fluoride risks in the areas and identify priority areas for further intervention

The output of the study will be used as an input for fluorosis mitigation efforts, used as a baseline for further risk assessment studies and information communication and education of endemic communities towards fluoride risks.

2.2 Objectives of the study

2.2.1 General Objective

To investigate the mean dietary fluoride intake and its mitigation in selected dietary areas

2.2.2 Specific Objectives

1. Investigate dietary fluoride sources
2. Estimate mean dietary fluoride intake by school child and biological mother
3. Assess fluorosis dietary risk factors
4. Assess dental and skeletal fluorosis level in selected areas in school age child and biological mother
5. Estimate urinary fluoride level as compared to ingested fluoride in school age child and biological mother
6. Investigate community attitudes on fluorosis and ingested fluoride mitigation
7. Investigate mitigation of ingested fluoride through Ca or moringa supplementation using rats
8. Investigate mitigation of ingested fluoride through Ca or moringa blended wheat bread in mothers using urinary fluoride as biomarker
9. Validation of fluoride analysis methods in urine, feces, foods and water using known concentration of spiked fluoride and Certified Reference Materials (Fish and Plant tissue).

CHAPTER THREE: LITRATURE REVIEW

3.1 Fluoride

Fluorine ranks thirteen among the elements in order of abundance in the earth's crust. It is an inevitable part of the biosphere and human life (Waldbott, 1963). It is the most electronegative and reactive of all the elements and as a result, elemental fluorine does not occur in nature, but is found as fluoride mineral complexes (WHO, 2004). Fluorine combines directly with most elements and indirectly with few to form fluorides (Waldbott, 1963). Fluorides are ubiquitous in nature and are present in rocks, soil, water, plants, foods and air (Fawell *et al.*, 2006) as fluorspar (CaF_2), cryolite ($3NaF.AlF_3$) or apatite ($3Ca_3(PO_4).2Ca(F,OH,Cl)_2$ (WHO, 1994). Availability of fluoride from soil depends on the solubility of the fluoride compound, the acidity of the soil and the presence of water (WHO, 2011; Fung and Wong, 2001). Fluorides are ubiquitous in air, water and the lithosphere. Fluorine as an element is seventh in the order of frequency of occurrence, accounting for 0.06-0.09% of the earth's crust and occurs as fluoride, e.g. cryolite (Na3AlF6). Cryolite (used for the production of aluminium) and rock phosphates (used for the production of fertilizers) have fluoride contents up to 54%. Most of this fluoride is insoluble and not biologically available. Availability of fluoride from soil depends on the solubility of the compound, the acidity of the soil and the presence of water (SCHER, 2010; Fung and Wong, 2001).

Elemental fluorine, which is a member of the halogen family, is a pale yellow green, irritating gas with a sharp odour and atomic mass of 18.998. The term 'fluorine' is used to denote the element in any of its forms and 'fluoride' to denote free inorganic fluoride to which a fluoride ion-selective electrode (ISE) responds (O'Donnell, 1973) during fluoride analysis.

Fluorine / fluoride is not essential for human growth and development but it is considered to be beneficial in the prevention of dental caries (tooth decay). As a result, intentional fluoridation of drinking water and the development of fluoride containing oral care products (toothpastes and mouth rinses), foods (fluoridated salts) and supplements (fluoride tablets) have been employed in several parts of the world as a public health protective measure against tooth decay (SCHER, 2010). In many African and Asian countries, exposure to fluoride

comes from naturally occurring water, beverages, food, and to a lesser extent, from other environmental sources (Malde *et al.*, 1997). Fourteen countries in Africa, eight in Asia and the Middle East and six in the Americas face the problem of fluoride concentration above 1.5 mg F-/L in drinking water. Many of these countries are confronted with the problems of endemic dental and skeletal fluorosis (Frenckens *et al.*, 1990).

A body of scientific literature seems to suggest that fluoride intake may be associated with a number of adverse health effects. Dental and skeletal fluorosis are two well documented adverse effects of fluoride intake. Systemic effects following prolonged and high exposure to fluoride have also been reported and more recently effects on the thyroid, developing brain and other tissues, and an association with certain types of osteosarcoma (bone cancer) have been reported (NRC, 1993).

Individual and population exposures to fluoride vary considerably and depend on the high variability in the levels of fluoride in waters, and on individual dietary and oral hygiene habits and practices. The emerging picture from all risk assessments conducted on fluoride is that there exists a narrow margin between the recommended intakes for the prevention of dental caries and the upper limits of exposure. All assessments to-date call for continued monitoring of the exposure of humans to fluoride from all sources and an evaluation of new scientific developments on its hazard profile. Exposure assessment has been conducted by the European Food Safety Authority (EFSA) for setting upper tolerable intake levels (UL) related to concentration limits for fluoride in water (EFSA 2005, EFSA 2008a, EFSA 2008b) and dental products (SCCP 2009). A similar approach was taken by the United States National Academies of Science in its 2006 review of the United States Environmental Protection Agency's water standards for fluoride (NRC 2006).

There is a continuous controversy over the benefit of fluoride and, in particular, the practices of intentional water fluoridation in tooth decay prevention. This has led to several countries discontinuing drinking water fluoridation and others expanding it. Besides questioning the practice of intentional water fluoridation itself as being unnecessary or superfluous in the light of the high exposure to fluoride from other sources, opponents of water fluoridation have

pointed to reports showing that the health and environmental risks of the most commonly used fluoridating agents, silicofluorides (e.g. (hydro)fluorosilicic acid, sodium silicofluoride, disodium hexafluorosilicate or hexafluorosilicate or hexafluorosilicic acid), have not been properly assessed. Furthermore, they suggest that the presence of these chemicals in drinking water may cause adverse effects on the health of humans and exert possible exacerbating effects on fluoride disposition in bone. The debate over water fluoridation has prompted several questions from the European Parliament, from Ireland and the United Kingdom where intentional water fluoridation is still practiced (SCHER, 2010).

Fluoride, whether naturally present or intentionally added to water, food, consumer and medical products, is considered beneficial to prevent dental caries (tooth decay). However, the cause of dental caries is multi-factorial, and the causal factors include microorganisms in dental plaque, fermentable carbohydrates (particularly sucrose), time, the individual's health status and level of oral hygiene, which depends on socioeconomic and educational status (SCHER, 2010).

The concentration of fluoride in ground water in the EU is generally low, but there are large regional differences due to different geological conditions. Surface water usually has lower fluoride contents than ground water (most often below 0.5 mg/L) and sea water (between 1.2 and 1.5 mg/L). There are no systematic data on the concentration of fluoride in natural drinking water in EU Member States, but some data show large variations between and within countries, e.g. Ireland 0.01-5.8 mg /L, Finland 0.1- 3.0 mg/L, and Germany 0.1-1.1 mg/L (SCHER, 2010).

WHO established a guidance value for naturally occurring fluoride in drinking water of 1.5 mg/L based on a consumption of 2 L water/day, and recommended that artificial fluoridation of water supplies should not exceed the optimal fluoride levels of 1.0 mg/L (WHO 2006). In Europe, only Ireland and selected regions in the UK and Spain currently fluoridate drinking water at concentrations ranging from 0.8 to 1.2 mg/L (Mullen 2005).

Fluoride is widely distributed in the atmosphere, originating from the dust of fluoride containing soils, industry and mining activities, and the burning of coal. The fluoride content in the air in non-industrialized areas has been found to be low and is not considered to contribute more than 0.01 mg/day to the total intake. Fluoride intake from food is generally low, except when food is prepared with fluoridated water or salt. However, some teas (e.g. *Camellia sinensis*) represent a significant source of fluoride intake (Malde *et al.*, 1997; Zerabruk *et al.*, 2010). Fruit and vegetables, milk and milk products, bread and cereals contain between 0.02-0.29 mg/kg (EFSA 2005). Recently, EFSA (2008a, 2008b) has permitted CaF_2 and Na_2PO_3F as a source of fluoride in food supplements.

Dental products (toothpaste, mouthwashes and gels) contain fluoride at different concentrations up to 1,500 mg/kg (1,500 ppm). The mean annual usage of toothpaste in EU Member States in 2008 was 251 mL (range 130-405 mL) per capita. The extent of systemically available fluoride from toothpaste depends on the percentage of toothpaste swallowed per application (SCHER, 2010).

An upper tolerable intake level (UL) of 0.1 mg/kg BW/day for fluoride has been derived by the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) (EFSA 2005) based on a prevalence of less than 5% of moderate dental fluorosis in children up to the age of 8 years as the critical endpoint, i.e. 1.5 mg/day for children 1-3 years of age, and 2.5 mg/day for children aged 4-8 years. For adults, an UL of 0.12 mg/kg BW/day was based on a risk of bone fracture, which converts on a body weight basis into 7 mg/day for populations aged 15 years and older, and 5 mg/day for children 9-14 years of age. Tolerable upper intake levels for fluoride have not been established for infants. For infants up to 6 months old, the UK Department of Health (UK DoH 1994) concluded that 0.22 mg F/kg BW/day was safe.

3.2 Chemistry of Fluoride

3.2.1 Fluorine

Fluorine, F_2 (mp, –218°C; bp, –187°C), is a pale yellow gas produced from calcium fluoride ore by first liberating hydrogen fluoride with sulfuric acid, then electrolyzing the HF in a 4:1 mixture with potassium fluoride, KF, as shown in the reaction below

$$2HF + KF\,(\text{molten}) \longrightarrow H_2\,(\text{cathode}) + F_2\,(\text{anode})$$

Of all the elements, fluorine is the most reactive and the most electronegative (a measure of tendency to acquire electrons). In its chemically combined form, it always has an oxidation number of –1. Fluorine has numerous industrial uses, such as the manufacture of UF6, a gas used to enrich uranium in its fissionable isotope, uranium-235. Fluorine is used to manufacture uranium hexafluoride, SF6, a dielectric material contained in some electrical and electronic apparatus. A number of organic compounds contain fluorine, particularly the chlorofluorocarbons used as refrigerants and organofluorine polymers, such as DuPont's Teflon. Given elemental fluorine's extreme chemical reactivity, it is not surprising that fluorine gas is quite toxic. It is classified as "a most toxic irritant". It strongly attacks skin and the mucous membranes of the nose and eyes (Manahan, 2003).

3.2.2 Fluoride compounds

Hydrogen Fluoride

Hydrogen fluoride, HF (mp, –83.1°C; bp, 19.5°C), may be in the form of either a clear, colorless liquid or gas. It forms corrosive fumes when exposed to the atmosphere. The major commercial application of hydrogen fluoride is as an alkylating catalyst in petroleum refining. Pot room workers in the primary aluminum industry are exposed to levels up to 5 mg/m3 in the workplace atmosphere and exhibit elevated levels of F– ion in their blood plasma (Lund, 1997). Hydrogen fluoride in aqueous solution is called hydrofluoric acid, which contains 30 to 60% HF by mass. Hydrofluoric acid must be kept in plastic containers because it vigorously

attacks glass and other materials containing silica (SiO_2), producing gaseous silicon tetrafluoride, SiF_4. Hydrofluoric acid is used to etch glass and clean stone.

Both hydrogen fluoride and hydrofluoric acid, referred to collectively as HF, are extreme irritants to any tissue they contact. Exposed areas heal poorly, gangrene may develop, and ulcers can occur in affected areas of the upper respiratory tract. The toxic nature of fluoride ion, F–, is not confined to its presence in HF. It is toxic in soluble fluoride salts, such as NaF. At relatively low levels, such as about 1 ppm, used in some drinking water supplies, fluoride prevents tooth decay. At excessive levels, fluoride causes fluorosis, a condition characterized by bone abnormalities and mottled, soft teeth. Livestock are especially susceptible to poisoning from fluoride fallout on grazing land as a result of industrial pollution. In severe cases, the animals become lame and even die (Manahan 2003).

3.2.3 Hexafluorosilicic acid and hexafluorosilicate

Hexafluorosilicic acid and hexafluorosilicates are the most commonly used agents in drinking water fluoridation and it has been claimed that incomplete dissociation of these agents in drinking water may result in human exposure to these chemicals. The toxicology of these compounds is incompletely investigated. Recent studies have addressed the equilibrium of the free fluoride ion and fluorosilicate species in aqueous solutions over a wide concentration and pH range. In the pH-range and at the concentrations of hexafluorosilicates/fluoride relevant for drinking water, hydrolysis of hexafluorosilicates to fluoride was rapid and the release of the fluoride ion was essentially complete. Other hydrolysis products of hexafluorosilicate such as $Si(OH)_4$ are rapidly transformed to colloidal silica (Finney *et al.*, 2006). $Si(OH)_4$ is present naturally in drinking water in large quantities and is not considered as a risk. In summary, these observations suggest that human exposure to fluorosilicates due to the use of hexafluorosilicic acid or hexafluorosilicate for drinking water fluoridation, if any, is very low as fluorosilicates in water are rapidly hydrolyzed to fluoride, as illustrated in the following equation (Finney *et al.*, 2006):

$$H_2SiF_6(aq) + 6OH^-(aq) \text{ ----- } 6F^-(aq) + Si(OH)_4(aq) + 2H_2O(l)$$

Studies on Na_2SiF_6 and H_2SiF_6, compounds used to fluoridate drinking water, show a pharmacokinetic profile for fluoride identical to that of sodium fluoride (NaF) (Maguire *et al.*, 2005, Whitford *et al.*, 2008). It therefore seems unlikely that the rate and degree of absorption, fractional retention, balance and elimination of fluoride will be affected if these fluoride compounds are added artificially in low concentrations, or if fluoride is naturally present in drinking water (Whitford *et al.*, 2008).

Hexafluorosilicic acids used as fluoridating agents may contain some impurities. Concerns have been raised about several heavy metals present as low-concentration impurities in commercial hexafluorosilicic acid. The average concentrations of arsenic, mercury, lead and cadmium present in hexafluorosilicic acid are low. Therefore, fluoridation of drinking water only contributes to a limited extent to the total exposure to these contaminants. It has been claimed that fluoridated drinking water increases human exposure to lead due to solubilization of lead from drinking water pipes by formation of highly soluble lead complexes. The claim was based on relationships of drinking water fluoridation and blood lead concentrations observed in a case study (Coplan *et al.*, 2007).

Based on the available chemistry of fluoride in solution, the chemistry of lead and lead ions, and the concentrations of fluoride in tap water, it is highly unlikely that there would be an increased release of lead from pipes due to hexafluorosilicic acid. The added concentrations of hexafluorosilicic acid do not influence the pH of tap water, and do not form soluble lead complexes at the low concentrations of hexafluorosilicic acid present in the gastrointestinal tract after consumption of fluoridated drinking water (Urbansky and Schock 2000).

3.3 Fluoride in Health and disease

3.3.1 Essentiality of Fluoride

Many elements occur in living organisms in small amounts that early technologies were unable to detect their presence or measure their precise amounts (Tressaud and Haufe, 2008). Interest in trace elements in human and animal physiology began over a century ago with the development and advances in technology that allowed detection and measurement of traces of

a number of metal-containing compounds that were not previously suspected to be of biological significance (Churchill, 1931). During the 1930s, a wide range of nutritional disorders of humans and farm stocks were found to be caused by either deficiency or excessive intake of trace elements from the natural environment. Fluoride was recognized in those years by different researchers as a cause for mottling of tooth enamel when consumed during infancy and early childhood (Smith *et al.*, 1931; Iyengar *et al.*, 1978).

The criteria for identifying nutritionally essential trace elements have evolved extensively over the past 50 years. The definition of elements as essential is thus difficult and, depending on the definition of essentiality, not unanimous. Iyengar *et al.*, (1978) considered an element to be essential if

(1) The organism can neither grow nor complete its life cycle if the element is not available in sufficient quantity,

(2) The element cannot be wholly substituted by another element and

(3) The element has a direct influence on the organism and is involved in its metabolism.

Based on this definition, fluorine is not an essential element. Later Mertz (1987) established essentiality as being when a reduction of the element below the range of tolerable levels, better termed 'range of safe and adequate intake', results in a consistent and reproducible impairment of a physiological function. On this basis, fluorine was included in the list of essential elements because of its proven benefits for dental health and its suggested role in maintaining the integrity of bones.

The complex health effects of whole diets were amenable to scientific study only by separating foods into their components, which could be measured and their effects investigated individually. The gradual emergence of 'new' paradigms and their influence on nutrition research increased to the point where they began to modify the old paradigm. 'New', emerging paradigms were addressed by Mertz (1993) as follows:

(1) The concern with individual nutrients (dissection) is being complemented by a concern for balanced interactions within the whole diet (synthesis);

(2) The division of the elements into essential and toxic categories is slowly being replaced by the concept of the total dose–response curve and

(3) The dominating role of the concept of deficiency in determining nutritional requirements is gradually being complemented by a concern for the total health effects of elements.

In accordance with these paradigms, the Expert Consultation of the WHO, Food and Agricultural Organization and International Atomic Energy Agency accepted in 1996 a revised definition of essentiality that says: 'An element is considered essential to an organism when reduction of its exposure below a certain limit results consistently in reduction in a physiologically important function, or when the element is an integral part of an organic structure performing a vital function in that organism' (WHO/FAO/IAEA, (1996). This definition is not absolute; it depends on what is considered to constitute a "physiologically important function and consistent' functional impairment". Caries is not a result of fluoride deficiency; however, since the Expert Consultation considered resistance to dental caries to be a physiologically important function, the element fluorine was regarded as essential. Fluorine was, at the same time, classified into the group of potentially toxic elements, some of which may nevertheless have some essential function at low levels (WHO/FAO/IAEA, (1996).

It has to be recognized that, although fluorine should be probably regarded as an essential element, there is no evidence so far from human studies that overt clinical signs of fluoride deficiency exist. No specifically diagnostic clinical or biochemical parameters have been related to fluoride deficiency (SCSEDRI, 1997; WHO/FAO/IAEA, 1996). It must also be noted that an experimental diet completely free of fluoride, capable of provoking fluoride deficiency, is difficult to obtain. It is also difficult to prove that it is free of fluoride, because of methodological and analytical problems in determining fluorine at low levels (Tressaud and Haufe, 2008).

3.3.2 Fluoride in dentistry

The element fluorine in the form of the fluoride ion is well known to be associated with sound dental health (Murray *et al.*, 1991). Dean and his colleagues (Dean *et al.*, 1942) showed an

inverse relationship between mottled enamel and the occurrence of dental caries. Mottling had previously been shown to be caused by the presence of fluoride (Churchill 1931). But Dean and his colleagues came up with the conclusion that fluorine was responsible for reducing the incidence of dental caries (Dean *et al.*, 1942). They demonstrated that 1 part per million (ppm) of fluoride in drinking water was optimal, as it gave the greatest reduction in dental caries without also causing mottling of the teeth. As a result of this study, the condition of tooth mottling was renamed 'dental fluorosis', and the study paved the way for extensive fluoridation of domestic water supplies throughout the developed world (WHO, 1984).

Toothpaste containing fluoride became available in this period, and has also contributed to the reduction in the incidence of caries over the period covered. However, it is apparent that the reduction was greater in the cohort from the Republic of Ireland, who also had the benefit of fluoride in their drinking water. Similar findings have been reported in numerous similar studies worldwide demonstrating beyond reasonable doubt that there is an improvement in the dental health of populations with fluoridated drinking water (Paik *et al.*, 1997, Clarkson and McLoughlin, 2000).

Fluoridation of drinking water is only one of the ways that fluoride has been delivered to the community and improved dental health. Others include fluoridated table salt, fluoridated milk, fluoridated dentifrices, topical fluoride gels, varnishes, and mouth-rinses, and fluoride-releasing dental restorative materials. An understanding of the underlying chemistry involved is critical to an appreciation of the clinical function of fluoride. Researchers have now reached the point where the effect of fluoride on the mineral phase of the tooth, hydroxyapatite, is reasonably well understood, and can be used as starting point for considering the contribution of fluoride to dental health (CDC, 2001).

3.3.3 Cariostatic Effect of Fluoride

The cariostatic action of fluoride on erupted teeth of children and adults is due to its effects on the metabolism of bacteria in dental plaque and on the dynamics of enamel de- and remineralization during an acidogenic challenge (Marquis, 1995; FNB, 1997). Plaque fluoride concentrations are directly related to the fluoride concentrations in and frequencies of

exposure to water, beverages, foods, and dental products. Fluoride can be deposited in plaque by direct uptake from these sources as well as from the saliva and gingival crevicular fluid after ingestion and absorption from the gastrointestinal tract. Its effects on plaque bacteria involve inhibition of several enzymes, which limits the uptake of glucose and thus reduces the amount of acid produced and secreted into the extracellular plaque fluid (Kanapka and Hamilton, 1971; Marquis, 1995). These effects attenuate the pH drop in plaque fluid that would otherwise occur and, hence, the severity of the acidic challenge to the enamel (Birkeland and Charlton, 1976; FNB 1997).

According to Ten Cate (1990), the effects of fluoride on the processes of enamel demineralization and remineralization in erupted teeth include:

(1) a reduction in the acid solubility of enamel;

(2) promotion of remineralization of incipient enamel lesions, which are initiated at the ultra-structural level several times each day according to the frequency of eating or drinking foods containing carbohydrates metabolizable by plaque bacteria;

(3) increasing the deposition of mineral phases in plaque, which, under acidic conditions produced during plaque metabolism, provide a source of mineral ions (calcium, phosphate, and fluoride) that retard demineralization and promote remineralization; and

(4) a reduction in the net rate of transport of minerals out of the enamel surface by inducing the reprecipitation of fluoridated hydroxyapatite within the enamel.

These various mechanisms underlying the protective effects of fluoride on the erupted teeth of children and adults require frequent exposures to fluoride throughout life in order to achieve and maintain adequate concentrations of the ion in dental plaque and enamel. Following the discovery of the link between fluoride and reduced incidence of dental caries community fluoridation programs were instituted in America. By the early 1950s, it was apparent that dental caries had been reduced by around 50% in those areas that had a fluoridated water supply (Clarkson and McLoughlin, 2000; Ast *et al.*, 1956). Since then, different countries across the world have moved to provide fluoridated water as standard to their populations.

However, due suspected negative health effect of fluoride its exposure has been closely regulated. The current estimates of optimum level of exposure to fluoride are between 0.05 and 0.07 mg/kg of body weight (Burt 1992). Some researchers have suggested that even lower levels, for example, 0.03–0.04 mg/kg, are appropriate, although these are lower than those typically aimed for in public health programs (Fejerskov *et al.*, 1982). Fluoride is especially important for children to prevent caries. However, it is important to regulate intake, as excessive exposure to fluoride can result in fluorosis. There are several sources of fluoride available to typical children in the developed world, including dentifrices, milk and other foodstuffs, as well as drinking water (Clarkson and McLoughlin, 2000). All these sources need to be taken into consideration when setting the level of fluoride to be added to public drinking water supplies (WHO, 2011).

Current levels of fluoride in water were based on findings of Galagan and Vermilion (1957) from the late 1950s, though they have since been modified downwards slightly. Thus, the World Health Organization recommends levels of fluoride between 0.5 and 1.0 ppm ((WHO, 2011; Heller *et al.*, 1999). Levels of water consumption do not vary particularly widely with climate, but are only about 20% higher in regions with higher average air temperatures (Heller *et al.*, 1999). Also, they vary throughout the year, again reflecting the local air temperature, with water consumption being greater in warmer conditions (Heller *et al.*, 1999). Consequently, there is no need to adjust the overall level of fluoride to take account of local climate and local variations in patterns of water consumption.

Dental caries is one of the most common diseases in humans, and is mediated by bacteria, mainly Streptococcus mutans (S. mutans). It has been estimated to affect between 60 and 90% of school children in the developed world and the majority of adults (Petersen and Lennon, 2004), and is consequently a major public health concern. In addition to the developed world, it is the most widespread oral disease in many Asian and Latin American countries, though it currently appears to be less of a problem in Africa (Wondwosen, 2004b). However, changes in living conditions and increases in sugar consumption within a changing diet suggest that, in

Africa too, the incidence of caries will increase, and that it will become a major public health issue (Tressaud and Haufe, 2008).

The presence of S. mutans and other cariogenic bacteria contributes towards the formation of a biofilm known as dental plaque, and their metabolism of fermentable carbohydrates in the diet leads to the formation of acids (Featherstone, 1999). Dental caries has been described as a complex imbalance in physiologic equilibrium between tooth mineral and biofilm (Fejerskov and Nyvad, 2003). Biofilms imply the involvement of microbiological species (Fejerskov, 2004), but the key concept included within this definition is that the bacteria involved are native to the body, not a group of specific invasive bacteria causing infection (Fejerskov, 2004).

The resident microbes within the mouth readily form biofilms on teeth. A biofilm consists of a population of bacteria coexisting in an orderly structure at the interface of a solid and a liquid within a biofilm, bacteria living in colonies encapsulated in a matrix of extracellular polymer. Oral biofilms are known to vary extensively in structure throughout the colony, with regions of densely packed microorganisms surrounded by open water channels. Each type of bacteria exists in reasonably defined environments which are influenced by surrounding cells, distance from the outer surface and local structure, all of which influence availability of nutrients and ambient pH (Fejerskov, 2004).

As far as caries is concerned, lesions develop where biofilms accumulate and are allowed to mature for prolonged periods of time. This includes occlusal surfaces, interproximal areas and along the margin of the gingiva. Once a frank cavity forms, it becomes an altered environment that becomes populated with biofilm organisms capable of adapting to the reducing local pH. In such cavities, the metabolism of the biofilm and the diffusion of substances through it become different from those of the biofilms over either sound or inactive caries (Fejerskov *et al.*, 1992).

The bacteria within a biofilm produce organic acids, the proportions of which vary depending on whether caries is active, that is, progressing through the tooth, or arrested; and also with

depth into the cavity, whether present in enamel or dentine (Hojo *et al.*, 1994). Active caries has been shown to have a low pH (Hojo *et al.*, 1991). There is destruction of both mineral and organic phase of the tooth as a result of the action of these acids, though most damage is done to the mineral phase (Featherstone, 1999). Its loss results in a greater amount of organic phases in the tooth, and this in turn becomes discoloured as a result of the action of these organic acids. The result is the yellowy-brown lesion characteristic of advanced dental caries (Hojo *et al.*, 1991).

When fluoride therapy was first used it was widely assumed that its effect was systemic. In particular, it was assumed to become incorporated into the tooth during development, forming the less acid-soluble fluorapatite rather than hydroxypatite (McClure and Likins 1951; McKay 1952). Studies showed that incidence of caries was lower in areas with fluoridated water and that fluoride concentration was higher in the surface of teeth of subjects in high fluoride areas (Chan *et al.*, 1989; Takeuchi *et al.*, 1996). However, over the last three decades or so, there has been a shift in our understanding, and it is now known that the principal effects of fluoride take place after eruption of the tooth (Hellwig and Lennon, 2004).

The modern view is that fluoride interferes with the progression of caries through a number of mechanisms, all of which apply after the tooth has erupted. These include the following:

- Inhibition of demineralization;
- Promotion of mineralization;
- Development of a mineral phase of enhanced acid resistance; and
- Damage to oral bacteria through inhibition of their enzymes (Featherstone, 1999).

Exposure to fluoride, by whatever means, leads to moderately elevated levels of fluoride in the saliva and the plaque. This is sufficient to help caries prevention by inhibiting demineralization and assisting remineralization. For many years, the process of caries was thought to be irreversible and to result in permanent loss of tooth material. This process eventually leads to the development of a cavity, and a considerable part of the dental professions' time is taken up with diagnosing and repairing such cavities. However, in recent years, it has become apparent that caries is not irreversible. Rather, as the process of tooth

maintenance represents a balance of demineralisation and remineralisation, so caries can be considered to be a shift in this balance, a shift, moreover, that can be reversed under the appropriate conditions (Tressaud and Haufe, 2008).

Re-mineralization occurs when partly dissolved crystals are induced to grow by precipitation of the mineral-forming ions; Ca^{2+} and $PO4^{3-}$. This is a natural process that occurs as a result of the concentration of these ions in saliva (Edgar and Highman, 1995) and it serves to oppose the demineralization effects of caries. The processes involved are complex and involve dynamic activity at the interface between the tooth, the saliva, the pellicle and the plaque. Fluoride plays a role in enhancing these processes, and though this is not the only contribution that fluoride makes to protect the tooth from caries, it is nonetheless an important one (Robinson, *et al*., 2000).

3.3.3.1 Possible antimicrobial effect of fluoride

There is some evidence that one potential effect of the release of fluoride is for it to act as an antimicrobial agent. It is known that millimolar concentrations of fluoride ion in water will affect a variety of activities in several types of cell (Simons, 1965). It does this by denaturing the enzymes. In bacteria, the most important enzyme which can be affected is enolase, and this is responsible for the conversion of 2-P-glycerate to 2-enol pyruvate in the glycolic pathway. Enolase is a magnesium-containing enzyme and, without Mg^{2+} ions, is deactivated. Fluoride, probably as monofluorophosphate ion, reacts preferentially with Mg^{2+}, removing it from the catalytic site in the enzyme and thereby inhibiting its activity (Hamilton, 1996; Hamilton and Ellwood 1985). Fluoride has other adverse effects on the biochemistry of bacteria, including inhibition of phosphatases (Luoma, 1980), which also contain magnesium. There is also evidence that fluoride generally has an adverse effect on bacterial growth (Yoon and berry 1979; Li and Bowden 1994).

Unfortunately, however, there is evidence that achieving sufficient fluoride levels in vivo to impair enzyme activity and inhibit bacterial growth is difficult. It may therefore be that such potential action of fluoride, for example on cariogenic bacteria such as S. mutans, has little or no part to play in practical caries prevention (Kaminsky *et al.*, 1990). In addition, it seems that

S. mutans can adapt to the presence of fluoride in such a way that its cariogenic potential is reduced. In in-vitro tests in rats, fluoride resistant strains of S. mutans were found to produce cariogenic acids more slowly than normal strains. However, this effect has not been demonstrated in human subjects (Van Loveren 1990).

3.3.3.2 Interaction of fluoride with hydroxyapatite

Fluoride is a powerful calcium-seeking element and can interfere with the calcified structure of bones and teeth in the human body, at higher concentration causing dental or skeletal fluorosis. This is because human and other animal bones are composed of hydroxyl apatite, which is an end-member in the apatite solid solution series. Therefore, fluoride exchanges readily with the OH- ion in the apatite structure increasing the brittleness and decreasing the solubility of the bone structure (Dissanayake, Chandrajith, 1999).

3.3.3.3 Basic chemistry of hydroxyapatite

The mineral phase of the tooth is predominantly hydroxyapatite, with the enamel containing a greater proportion than the dentine. This phase is very far from inert, but undergoes chemical exchange of ions with the surrounding saliva (Margolis *et al.,* 1988) driven by factors such as local fluctuations in pH of the plaque (Baelum *et al.,* 1994), and the presence of fermentable carbohydrates (Fejerskov *et al.*,1992). The major cariostatic effect of fluoride is that it influences these processes, even at very low concentrations and in particular it facilitates precipitation of calcium phosphate (Fejerskov, *et al.,* 1981). This is consistent with in vitro observations that fluoride is capable of inducing hydroxyapatite to form from solutions of calcium and phosphate ions. The inorganic composition of saliva is such that it is readily able to precipitate calcium phosphate (Koulourides *et al.,* 1965), and thus leads to remineralisation at the tooth surface. The presence of fluoride at slightly elevated concentrations enhances this ability as pH is lowered from the level of active to the level of arrested caries (pH 5.5 and above) (Koulourides *et al.*, 1965).

In addition, the presence of low concentrations of fluoride in saliva also has the effect of preventing dissolution of hydroxyapatite from enamel at low pH, an effect that has been shown to apply at values of pH as a low as 4.2 (Larsen *et al.,* 1976). Thus, it is the fluoride in

solution that has the effect of reducing solubility, rather than the fluoride in the mineral phase (Fejerskov *et al.*, 1981). This effect requires extremely small amounts of fluoride (Fejerskov, *et al* 1981; Lagerlof & Oliveby 1994) and has the effect of shifting the balance between demineralisation and remineralisation so that loss of the hard tissue is inhibited.

Nevertheless, fluoride does lead to a reduction in the solubility of hydroxyapatite in aqueous solution, even in the absence of trace levels of fluoride in solution, and hence can be seen to have an effect in the solid state as well (Arnold Jr., 1957). Apatites are complex and diverse materials which have the general formula $Ca_{10}(PO4)_6X_2$ (X = F, Cl, OH) and they represent a crystallographic system, in which there can be considerable replacement of species. Thus, with little or no change in the dimensions of the crystal lattice, there can be exchanges of OH^-, for F^-, Ca^{2+} for Sr^{2+}, and PO_4^{3-} for CO_3^{2-}; and all of these are known to occur in biological systems. Natural hydroxyapatite, for example, is often partially carbonate substituted (Penel *et al.*, 1998).

The incorporation of fluoride in place of hydroxyl groups is chemically straight forward (Kay *et al.*, 1964; Elliott, 1969) and show greater resistance to acid attack. This is partly due to the greater electronegativity of fluorine, which means that the electrostatic attraction between Ca^{2+} and F^- is greater than that between Ca^{2+} and OH^-. As a result, the fluoridated apatite lattice is more stable than hydroxyapatite (Kutnerian and Kuyper, 1957; Brown *et al.*, 1977; Young, 1975). It is also more crystalline (White and Nancollas, 1990).

Fluoride also brings about a change in composition in natural hydroxypatite, since it not only undergoes a simple exchange with hydroxyl ions but also promotes the formation of a phase containing less carbonate than the initial hydroxyapatite (Featherstone, 1999). Fluoride is taken up more readily by demineralised enamel than by sound enamel, which means its availability causes a 'self-healing' effect in the mineral phase of the hard tissue (Thylstrup *et al.*, 1979).

3.3.3.4 Exchange of Hydroxyl ion for fluoride: computational approach

The effect of fluoride on hydroxyl ion exchange as it affects the individual atomic layers of hydroxyapatite in a crystal has been modelled, using computation (De Leeuw, 2004). Computational methods of this type are able to calculate both the energy changes in exchanging hydroxyl ions for fluoride, and also to determine the expected distribution of the fluoride in the resulting crystal. These calculations provide interesting results about the overall process.

Substitution of hydroxyl ions by fluoride ions has been shown to be energetically favourable when that substitution takes place in the uppermost surface layer. It is highly exothermic, with an enthalpy change of 193 kJ/mol. Incorporation of fluoride into lower layers is less favoured. In the second layer, it yields 164 kJ/mol and in the third layer only 68 kJ/mol. Beyond third layer, there is almost no energy change associated with the exchange (De Leeuw, 2004). There is little or no driving force for fluoride to penetrate deep into the bulk of the hydroxyapatite crystal. These results show that it is relatively easy to replace the surface layer hydroxyl groups with fluoride ions, but go on to predict that fluoride will not penetrate much below about three atomic layers of the bulk crystal. As a result, whether fluoride is taken up by replacement of OH^- ions, or whether the mechanism of deposition involves re-precipitation of dissolved hydroxyapatite. The fluoridated form of apatite is found in the surface layers only (De Leeuw, 2004).

The effect of water on the surface has also been modelled De Leeuw, 2004), and the calculations have shown that surface layers of water are able to take up OH^- ions from hydroxyapatite more readily than F^- ions from the substituted apatite structure. Hence, F^- tends to remain in its place in the crystal lattice. The ease of dissolution can be determined from the calculated diffusion coefficients, which have been found to be respectively for OH^- and F^-, 5.74×10^{-9} cm^2/s and 0. By comparison, the calculated diffusion coefficient for molecules of water in the region close to the surface was 1.50×10^{-7}. This is similar to that for the hydroxyl ions, and demonstrates the significance of dissolution in water as part of the mechanism of movement of these ions. Sub-surface OH^- ions, by contrast, have been shown

to have a very low diffusion coefficient (1.23×10^{-12}), which suggests that these ions are almost completely immobile compared with surface layer ones (De Leeuw, 2004) .

In the presence of fluoride, calcium ions have been found to be more firmly bound than in pure hydroxyapatite (De Leeuw, 2004). This enhances the overall resistance to dissolution. Thus, the presence of a thin stable film of fluorapatite on the surface of hydroxyapatite crystals has two effects, namely;

(i) resistance to diffusion and dissolution of the anion and

(ii) firmer binding of calcium ions into the surface.

Both of these make the resulting apatite structure more resistant to dissolution, regardless of the pH of the external medium, and thereby increase the resistance of the mineral phase to the onset of caries.

Hydroxyapatite (with some carbonate inclusions) is the most stable of the possible calcium phosphate salts that can be formed under physiological conditions. However, it is not the most rapid one to form. Instead, octacalcium phosphate (OCP) will precipitate more readily than hydroxyapatite. This led Brown and his colleagues (1987) to propose that, as the kinetically favoured compound, OCP precipitates first, and then undergoes irreversible hydrolysis to a transition product; OCP hydrolyzate (Brown *et al.*, 1987). This hypothesis is consistent with the observation that enamel comprises hydroxyapatite crystals that have the long, plate-like morphology that is generally considered characteristic of OCP crystals (Aoba *et al.*, 1998). Overall, it seems that enamel crystals, with their elongated form, result from early precipitation of OCP, which forms a template on which hydroxyapatite units grow epitaxially (Iijima *et al.*, 1992; Miaka *et al.*, 1993). This leads to enamel mineralisation with the observed thin, ribbon-like structure of crystals. The role of fluoride in mineralisation process seems to be in promoting the conversion of OCP to hydroxypatite, and producing plate-like crystals of the more thermodynamically stable mineral. Fluoride is effective at promoting the formation of apatite lattice through a solid-state transformation of OCP (Mura-Galelli *et al.*, 1992).

Under neutral conditions, fluoride is also able to induce nucleation and growth of apatite crystals without the involvement of OCP (Mura-Galelli *et al.*, 1992). It is significant that in

severe cases of fluorotic enamel, ultra-structural studies have shown the occurrence of a proliferation of apatite nuclei, suggesting that the presence of fluoride may act to encourage precipitation of crystals of fluoroapatite (Yanagisawa, 1989).

The various findings about fluoride and its interaction with the hydroxyapatite at the molecular level show that the relationship is complicated and multifaceted. It promotes numerous desirable properties in tooth mineral, reducing solubility through action in both the saliva and in the mineral phase, it shifts the demineralization - remineralization equilibrium in favour of remineralization, and through its actions in the solid state, ensures that the kinetically favoured OCP is transformed into the more thermodynamically stable hydroxyapatite (Tressaud and Haufe, 2008).

3.3.3.5 Demineralization and remineralization behavior of the tooth surface

The first sign of caries in the enamel is the so-called 'white spot lesion', a feature which arises due to sub-surface demineralization (Edgar and Highman 1995). Although at this stage the structure appears reasonably intact, it may have lost up to 50% of its original mineral content (Silverstone, 1973). One of the difficulties in clinical dentistry is the fact that these early lesions may be difficult to detect, particularly when they are hidden in fissures and pits. By the time they are apparent, they may have progressed significantly and extended well into the dentine (Weerheijm *et al.*, 1992). Fluoride, in promoting remineralization, may contribute to a clinical problem with certain lesions. By encouraging only the surface layer to undergo remineralization, it may cause the underlying lesions to be concealed (Weerheijm, *et al.*, 1997). In such cases, remineralization of the sub-structure is inhibited because the mineralized surface that forms is relatively impermeable to calcium and phosphate ions, which consequently cannot penetrate the surface layer (Larsen and Richards, 2001).

It is now known that teeth undergo a continuous process of demineralization and remineralization, which is driven by changes in the plaque composition (Gao *et al.*, 2001). In the presence of fermentable carbohydrates plaque microorganisms generate characteristic organic acids, that is, lactic and acetic (Hojo *et al.*, 1991), and these diffuse through the

pellicle to the tooth surface and cause demineralization (Van Dijk *et al.*, 1983). Ions are then liberated from the mineral phase into this low pH liquid (Zhang *et al.*, 2000) and they diffuse outwards and re-precipitate at the surface layer of the demineralized lesion (Brown, 1974; Morino and Aoba, 1991). If this process is sufficiently rapid, there is a net loss of tooth mineral and irreversible cavity formation.

On the other hand, remineralization may occur at such a rate that the surface layer is retained. This requires continuous renewal of ions, either from among those newly solubilized or from those present in the saliva (Dreissens, 1982; Aoba, 2004).

Useful insights can undoubtedly be obtained by consideration of the likely chemical species present, and their behaviour at varying pH. Saliva is known to be metastable and supersaturated with respect to hydroxyapatite (Dreissens, 1982), which is further evidence that the purely thermodynamic approach is suspect. It does mean though that there is a significant driving force for remineralisation, as the supersaturated saliva approaches equilibrium with the consequent precipitation of hydroxyapatite. The development or arrest of a carious lesion is therefore dependent on the frequency and duration of the remineralisation and demineralization processes, and also on their respective rates. These in turn depend on factors such as sucrose consumption, salivary flow, oral hygiene (Nikiforuk, 1985) and fluoride exposure (Fejerskov *et al.*, 1996).

3.3.3.6 In Vitro Studies of Effect of Fluoride on Tooth

There has been a large volume of work on the chemistry of the effect of fluoride on caries. Typically, studies have employed an artificial caries medium, and exposed extracted teeth to acidified buffer solutions or gels adjusted to pH 4–6 using lactic acid or acetic acid. Such studies have demonstrated that fluoride reduces the rate of formation of carious lesions in vitro (Spierset *et al.*, 1963) and also that the appearance of the resulting lesion is modified (Larsen, and Fejerskov, 1977). Chemical analysis of white spot lesions have shown to contain more fluoride than the surrounding enamel. The result suggests that fluoride has a greater affinity for demineralised regions. As a result, precipitation of hydroxyapatite is accelerated, particularly around the edges of the lesion. Decrease in pH reduces the OH^- concentration,

leading to relatively enhanced F^- concentration which in turn increases the rate of fluoride uptake as a consequence of mass action effects (Ten Cate, 1997; Sakkab *et al.*, 1984).

3.3.3.7 Fluoride in reducing hypersensitivity

Fluoride is also employed clinically to reduce hypersensitivity in patients. It may be employed at low levels, that is, less than 1%, in solutions, and as such it is effective in reducing reported pain levels in patients experiencing hypersensitivity (Thrash *et al.*, 1992). Fluoride-containing varnishes have also been shown to reduce such pain (Ritter, *et al* 2006), though not to a greater extent than non-fluoridated varnishes. Fluoridated toothpastes also seem to have a positive effect on reducing hypersensitivity. The action of fluoride is linked to the ease of precipitation of CaF_2 through interaction with saliva, leading to sealing of structural imperfections in the tooth surface. This reduces the ease with which hot or cold liquids can diffuse into the tooth and eventually through to the pulp, causing the sensation of pain. Although not the most important effect of fluoride, the effect on hypersensitivity is further evidence of generally beneficial role of this element. More important is its effect of caries (MacCarthy, 2004).

3.3.4 Fluoride in Bone health and Osteoporosis

Fluoride stimulates osteoblast activity and can replace hydroxyl ions in the hydroxyapatite structure of bone. The substitution results in bone with increased crystalline size but decreased elasticity and quality. Although the compression strength of fluoride-enriched bone is greater, the tensile quality may decline. An association between increased bone density on radiographs and naturally occurring high fluoride in drinking water, at concentrations between 4 to 5.8 mg/L, was observed in an early studies (Shils et al., 2006). When used in high doses as a therapeutic agent for osteoporosis, fluoride increases spinal bone density, but the effect on fracture incidence is less clear (OSC, 2003). Several researchers applied sodium fluoride therapy hopping to be effective in preventing fractures in postmenopausal women with osteoporosis. However, despite consistent and significant increases in spinal bone mineral density (BMD), there is no evidence for a reduction of vertebral or non-vertebral fractures. Fluoride has the ability to maintain the level of BMD or yield slight increases at the femoral

neck. Increases in spinal BMD with fluoride therapy in glucocorticoid- induced osteoporosis were too small to show a significant anti-fracture effect (OSC 2003; Pitt and Berry 1991).

Although fluoride increases bone mass, the newly formed bone may have reduced strength. In their finding Reggs *et al* (1990) found increases in median bone mineral density ($p<0.0001$) in the lumbar spine (predominantly cancellous bone), in the femoral neck, in the femoral trochanter but the bone mineral density decreases in the shaft of the radius (cortical bone). They concluded that fluoride therapy increases cancellous but decreases cortical bone mineral density and increases skeletal fragility (Reggs *et al* 1990).

The use of fluoride for the treatment of established vertebrai osteoporosis in adults with the symptomatic crush fracture syndrome is approved for use in 8 European countries. This treatment was suggested by the low prevalence of osteoporosis in some areas where the drinking water contained moderately high concentrations of fluoride' and by the enormously increased bone density characteristic of fluorosis. A wealth of evidence and some controversy regarding the use of fluoride for bone health (Pitt and Berry, 1991) exist. Fluoride is known to stimulate osteoblasts in culture. Its use has been associated with increase in alkaline phosphatase activity and it stimulates both collagen synthesis and calcium deposition. High concentrations may exert a toxic effect on osteoblast function (Whitford & Pashley, 1984).

3.3.5 Potential systemic effects

Fluoridation of drinking water causes every member of the population to be exposed to fluoride treatment, though its principal benefits are for children. This is the basis for one of the widely used arguments against fluoridation of water supplies. The concern which underlies this argument has led to certain public organizations across the world ceasing to fluoridate the water. An example of this was the Gold Coast Council in Queensland, Australia, which stopped fluoridation in 1979 (Demos *et al*., 2001).

Another much discussed concern is the potential effect on bone, with conditions such as skeletal fluorosis, osteosarcomas, osteoporosis and greater incidence of fractures. Animal studies have demonstrated that bone strength decreases with high doses of fluoride. In rats,

this required at least 50 ppm/day or 0.23 mg/kg/day (Turner *et al.*, 1992), with at least three months treatment needed before loss of strength was significant (Turner *et al.*, 1995). In rabbits, high dose rates of fluoride were similarly required in order to produce an effect. By contrast, exposure to low amounts of fluoride increased bone strength in rats without increasing femoral bone density (Turner *et al.*, 1992). For comparison, consuming a typical amount of water (1 L/day) at a concentration of 1 ppm provides a dose of 0.02 mg/kg/day for a 60-kg person (Demos *et al.*, 2001).

Hip fracture has been reported to increase in two studies, respectively after 4 years (Jacobsen et *al.*, 1992) and 7 years (Danielson *et al.*, 1992) of exposure to fluoridated water. However, other studies have shown either no effect after 6 years or a reduction in incidence. In those studies inability to control such variables as age, races, poverty, activity levels or overall health status were mentioned (Suarez-Almazor, *et al.*, 1993; Jacobsen *et al.*, 1993).

Clinical trials have been concentrated on bone mineral density, because this parameter is an acceptable measure of bone mass. It is sensitive to the occurrence of osteoporosis and correlates well with the likelihood of bone fracture in patients affected by osteoporosis. Bone mineral density is known to increase in childhood until the age of 40 after which it then declines (Lentle, 1998; Mirsky and Einhorn 1998). In women in the years immediately following the menopause, it may sharply reduce below the young adult mean value where the condition is defined by the WHO as osteoporosis (Lentle, 1998; Mirsky and Einhorn, 1998).

Daily doses studied have ranged from 9 to 22.6 mg of fluoride over time periods of 1–4 years (Demos *et al.*, 2001). These trials were particularly concerned with the use of slow-release NaF (Pak *et al.*, 1995; 1996) or sodium monofluorophosphate preparations and they generally led to reductions in the incidence of bone fracture. In addition, they typically caused increased bone density at the neck of the femur, the femoral condyle and the lower spine (Sebert *et al.*, 1995). These results confirm the findings of animal studies that lower doses have greater beneficial effects. Overall, they are consistent with the general conclusion from a large number of studies in different regions and with highly varied populations that fluoridated

water has no adverse effect on bone. On the contrary, there is clear evidence that it is beneficial (Demos *et al.*, 2001).

3.4 Excess intake of Fluoride: Fluorosis

Chronic and acute toxicity of inorganic fluorides were first distinguished by Roholm (1937), who was the originator of modern fluoride research. Chronic toxicity is the result of continuous or repeated exposure of an organism to a toxic substance. Acute toxicity involves harmful effects in an organism through a single or short-term exposure to a toxic substance.

3.4.1 Chronic toxicity

Fluoride is a cumulative toxin, which accumulates in mineralized tissues, notably in the lattice of bone and tooth crystals (SCSEDRI, 1997; WHO/FAO/IAEA, 1996). The biological effects in humans due to chronic fluoride ingestion depend not only on the total dosage and duration of exposure, but also on associated factors such as nutritional status, functional status of the renal tissue and interaction with other trace elements. The effect of fluorine is cumulative, so that less serious consequences occur early in the natural course of disease. Whatever may be the type of fluorine exposure, the clinical picture in chronic poisoning occurs in a phased manner (Mertz, 1987).

The primary adverse effects associated with chronic, excess fluoride intake range from mild dental fluorosis to crippling skeletal fluorosis as the level and period of exposure increases. Other effects, including hypersensitivity reactions, renal insufficiency, immunological effects, possible association with repetitive strain injury, birth defects and cancer have been observed and discussed (WHO, 2002; Mertz, 1987; WHO, 1984; Marshould, 1990; Harrison, 2005). There is more of a threat, however, to the higher concentrations due to the severity of the disease posed by such levels within the water. The severity depends upon the amount ingested and the duration of intake (WHO, 2004).

3.4.2 Fluorosis

Fluorosis is regarded as the most serious adverse effect of exposure to relatively low doses of fluoride (McDonag, 2000). In extreme cases, fluorosis manifests itself as brown mottling of the enamel, and also results in overall yellowing of the teeth, and a greater degree of brittleness. It occurs as the result of excessive consumption of fluoride above optimum level (Whitford, 1997). As ever, the dose critically determines whether there will be any adverse effects, and this depends inter alia on the amount taken in, the proportion absorbed by the body and the weight of the patient. In response to these considerations, the medical profession tends to advise mothers not to make up infant feeds from fluoridated water, though there is as much concern with the fact that ingested fluoride promotes removal of calcium from the body as CaF_2 in the feces as with any adverse effects of retained fluoride. Another group who are advised against consuming fluoridated water are patients on kidney dialysis (McDonag 2000). According to Susheela (1999) some groups of population namely pregnant and lactating women, young children, malnourished children, people with low intake of calcium, people with cardio-vascular and kidney problems and those who involved in hard manual labor in hot climates are more susceptible to the poisonous effects of fluoride than others.

3.4.2.1 Dental fluorosis

The most sensitive effect of fluoride exposure in humans is dental fluorosis. Dental or enamel fluorosis is an irreversible dose–response effect caused by fluoride ingestion during the pre-eruptive development of teeth (Dean 1942). Dental fluorosis in a mild form is a cosmetic effect that ranges in appearance from scarcely discernible to a marked staining or pitting of the teeth in severe forms (Whitford, 1997). The pre-eruptive maturation of crowns of the anterior permanent teeth, which are of most concern aesthetically, is complete and, together with the risk of fluorosis, is over by the age of 7–8 years (Fejerskov,1977; Ishii & Suckling,1991). Therefore, the most sensitive population to dental fluorosis is children under the age of eight particularly during the pre-eruptive formation and maturation of enamel in teeth (Liang *et al.*, 2006). Excessive fluoride intake by peoples older than 7 years will not cause dental fluorosis (Stefanie *et al.*, 2009). Therefore, fluoride intake up to the age of 7–8 years is of most interest. Although it is usually the permanent teeth that are affected, occasionally the deciduous teeth may be also involved. Dental fluorosis is more prevalent in

children fed formula than in breast-fed children. The earliest signs of dental fluorosis can be seen as small white lines across the entire enamel surface (WHO, 2003).

Fluorosis affects the enamel of the tooth, causing it to become hypomineralised. This is detected as visual changes in the opacity, and it is only in extreme cases that this leads to an adverse appearance as mottling of the tooth surface (Thylstrup and Fejerskov 1978). The severity of the discoloration depends on the dose of fluoride, its duration and timing of consumption. Teeth are most susceptible to the adverse effects of excess fluoride at the transition and early maturation stages of enamel development (Bardsen and Bjorvath 1998). The timing of these stages varies with the type of tooth (Evans and Stamm, 1991). For the most prominent teeth, the upper central incisors, the most sensitive period appears to be 13-24 months, with some indication that the timing varies slightly for boys and girls (Bardsen and Bjorvath 1998; Evans and Stamm, 1991).

The risk of fluorosis is only of concern for children below about 8 years of age, because enamel can no longer be affected once pre-eruptive maturation has occurred (Jackson *et al.*, 1999). As far as cosmetic effects are concerned, the critical age is somewhat younger because at this age the central incisors are undergoing development, and hence are at a stage that makes them susceptible to fluorosis. For children at the age likely to be affected, the main sources of fluoride are drinking water, processed food and beverages, toothpaste and other dental products (i.e., tablets or drops).

In its mildest form, fluorosis appears on the enamel surface as chalky, lace-like marks, and these may not be apparent to the casual observer (Thylstrup and Fejerskov, 1978). As severity increases, over half of the enamel surface may appear white and opaque. In its most severe form, fluorosis causes the enamel to become pitted and brittle. Despite this embrittlement, fluorosis rarely causes the teeth to suffer from severe mechanical problems, so the condition is considered to be an aesthetic rather than a functional problem by some researchers and clinicians (Fejerskov *et al.*, 1994). Both moderate and severe fluorosis may be accompanied by the development of a brown discoloration (Bowen, 2002).

In general, moderate and severe fluorosis is rare except in naturally fluoride contaminated areas like Asia and East Africa (Teklehaimanot *et al.*, 1987, Sucheela, 2002). However, the mildness of the fluorosis detected is associated with only very slight changes in the appearance of the teeth, which suggests that, even at these levels, it is not a major public health problem (Griffen *et al.*, 2002). Nonetheless, it is appropriate to ensure that parents or guardians of children continue to receive sound advice on safe levels of fluoride for those in their care to be exposed to and, since the cariostatic effect of fluoride is known to occur well after enamel formation during tooth development, treatment to reduce caries should concentrate on those measures that carry the lowest possible risks of fluorosis in countries which have fluoridation program (Fejerskov *et al.*, 1994).

In most cases, fluoridated toothpastes are acceptable substances for use, but even for these products, there is some risk of fluorosis. For example, children who began using them before the age of 2 years were shown to be at higher risk of developing fluorosis than children who do not use it at all (Pendrys *et al.*, 1994). However, the relative importance of the various factors that govern exposure to fluoride from this source (age of starting to use fluoridated toothpastes, amount used and frequency) is not known. One contributor to this risk of fluorosis in children is the lack of control in the swallowing reflex, particularly in children younger than 3 years of age. Children are also known to like the taste of toothpaste, and hence to swallow it deliberately (Simard*et al.*, 1991).

A small toothbrush of the size appropriate for use by a child holds in the region of 0.75–1.0 g of toothpaste and an individual blob of toothpaste provides between 0.75 and 1.0 mg of fluoride. It has been estimated that children below the age of six swallow a mean of 0.3g of toothpaste per brushing and this may be sufficient to lead to ingestion of enough fluoride to cause the mottling associated with fluorosis (Levy, 1993).

Determination of dental fluorosis

The symptoms of fluorosis are easy to recognize. The clinical features of mild dental fluorosis vary from thin white striations across the enamel surface to white flecks or small pits on the enamel of the teeth. With increasing severity, the white areas merge and loss of enamel

surface can occur. Discrete and confluent pitting of the surface of the teeth is seen in severe cases. The loss of enamel involves only the surface and not the full thickness of the enamel. Mottled enamel has high protein content, resulting in increased porosity; as a consequence, brownish discoloration of the enamel may occur due to uptake of color from diet into the porous enamel. Mild dental fluorosis has no effect on tooth function and may render the enamel more resistant to caries (SCSEDRI, 1997). This type of fluorosis is not readily apparent to the affected individual or casual observer and often requires a trained specialist to detect it. In contrast, the moderate and severe forms of dental fluorosis are generally characterized by aesthetically (cosmetically) objectionable changes in tooth color and surface irregularities.

Several methods have been developed for quantifying dental fluorosis. The most commonly used method is Dean's index (Dean 1934), which classifies fluorosis on a scale of 0 to 4 as follows:

Class 0: No Fluorosis;

Class 1: Very Mild Fluorosis (opaque white areas irregularly covering 25% of the tooth surface);

Class 2: Mild Fluorosis (white areas covering 25–50% of the tooth surface);

Class 3: Moderate Fluorosis (all surfaces affected, with some brown spots and marked wear on surfaces subject to attrition)

Class 4: Severe Fluorosis (widespread brown stains and pitting)

The average score of the two most severely affected teeth is used to derive the classification. Other commonly used methods to rate dental fluorosis include the Thylstrup-Fejerskov Index (TFI) (Thylstrup and Fejerskov 1978) and the tooth surface index of fluorosis (TSIF) (Horowitz *et al.*, 1984). Unlike the Dean's index, the TFI and TSIF use all tooth surfaces to develop the final index score (Wondwosen *et al.*, 2004b).

3.4.2.2 Skeletal fluorosis

Skeletal fluorosis can be defined as excessive deposition of fluoride in bone. This is a pathological condition that is by far the most important aspect of chronic exposure to elevated

levels of fluoride, either by inhalation or by ingestion (Shashi et al., 2008). In adults; stiffness of the back and neck muscles, unable to bend forward and to stand straight are some of the indicators of skeletal fluorosis. On the other hand, signs and symptoms of skeletal fluorosis in children include; pain in the lower limbs, knock knee, bow leg and anterior bowing of the lower limb bones (Tekle-Haimanot, 1990).

The skeletal deformities may be associated with or accentuated by nutritional deficiencies or even malnutrition and hard manual work or, possibly, other conditions found in areas of long-term social and nutritional deprivation (WHO 1994). The situation is specific also for populations consuming large volumes of water, such as athletes or people with certain medical conditions or in some tropical areas where, due to higher temperatures, water consumption is increased. Skeletal fluorosis exhibits several stages. According to NRC (2006), skeletal fluorosis is categorized into one of four stages: a preclinical stage and three clinical stages that increase in severity. Two pre-clinical asymptomatic changes are characterized by slight, radiographically detectable increases in bone mass. An early symptomatic stage is characterized by sporadic pain and stiffness of joints, arthritic symptoms, slight calcification of ligaments and increased osteosclerosis of cancellous bones, sometimes accompanied by osteoporosis of long bones. The most severe stage (clinical stage III) historically has been referred to as the "crippling" stage. Crippling skeletal fluorosis is characterized by marked limitation of joint movements, considerable calcification of ligaments, crippling deformities of the spine and major joints, muscle wasting and neurological defects associated with compression of the spinal cord (Kaminsky *et al.*, 1990). NRC (2006) concluded that "the weight of evidence supports the conclusion that lifetime exposure to fluoride at drinking water concentrations of 4 mg/L and higher is likely to increase fracture rates in the population.

According to the US EPA (2010), however, although there are a large number of epidemiological studies available, the data are such that it is difficult to determine a clear exposure–response relationship. One possible feature of fluorosis is bone fracture, although some studies have reported a protective effect of fluoride on fracture. The NRC analysis determined that severe dental fluorosis appears to occur at a lower dose than stage II skeletal

fluorosis and/or bone fractures (NRC, 2006).

Epidemiology of skeletal fluorosis

Endemic crippling skeletal fluorosis is confined in temperate climates to individuals exposed continuously over many years to very high levels of fluoride; these cases are associated with industrial situations (Tekle-Haimanot 1990; Shashi *et al.*, 2008), with unusually high levels of fluoride in drinking water (e.g., 10 mg/L) (Ethiopia, Uganda and India) or the use of high fluoride coal for cooking and drying foodstuffs indoors (WHO, 1994, 2002).

Most data on the occurrence of skeletal fluorosis in occupationally exposed workers have come from older studies. Roholm (1937) estimated that, for cryolite workers, the effective toxic daily fluoride dose lies very probably between 0.20 and 0.35 mg/kg (equivalent to 14–25 mg for a 70-kg man). This intake for 10–20 years causes mild to severe signs of osteosclerosis. Hodge and Smith (1977) reviewed older studies involving aluminum smelter workers, in which the number of workers examined was usually small and quantitative data on exposure to airborne fluoride were not always provided. They concluded that the incidence of detectable osteosclerosis was often high when the levels of fluoride in the air exceeded 2.5 mg/m^3 and/or levels of fluoride in the urine of these workers were greater than 9 mg/L. At airborne concentrations below 2.5 mg/m^3 and levels in the urine below 5 mg/L, years of exposure in potrooms produced no osteosclerosis.

Most epidemiological research has indicated that an intake of at least 10 mg/day for 10 or more years is needed to produce clinical signs of the milder forms of osteosclerosis. Water fluoride concentrations of 4–8 mg/L in temperate climates have not been found to be associated with any signs or symptoms of skeletal fluorosis (WHO, 1994). This data should be regarded with scepticism in view of reports from a number of developing countries that endemic skeletal fluorosis occurs in individuals whose drinking water contains more than 6 mg/L of fluoride (WHO, 1994).

In an epidemiological study in China the relationship between fluoride intake via drinking-water and all other sources, followed a U-shaped dose response with higher rates of fracture at

very low intakes below 0.34 mg/L and high intakes above 4.32 mg/L (total intake 14 mg per day) (Li *et al.*, 2001). It was concluded by the IPCS (2002) that for a total intake of 14 mg per day there is a clear excess risk of skeletal adverse effects and there is suggestive evidence of an increased risk of effects on the skeleton at total fluoride intakes above about 6 mg per day. However, skeletal fluorosis has been reported in India in a village with average fluoride concentration in drinking water as low as 0.7 mg/L, with range 0.4–1.4 mg/L (Jolly, 1973). Although the disease is uncommon at such low concentrations, evidence of skeletal fluorosis, with severe clinical manifestations, has been also reported in at least 9 studies from 5 countries, where water supplies contain fluoride naturally in the range 0.7–2.5 mg/L (Diesendorf, 1990).

Determination of skeletal fluorosis

Skeletal fluorosis rate can be screened in endemic areas using clinical symptoms and physical exercise (Figure 2) as developed by Susheela *et al.*, (2002) and used by Shashi & Bhardwaj (2008). The severity of muscle stiffness due to fluorosis skeletal fluorosis amongst the different age groups can be determined using the following prescribed physical exercises with focused communities in endemic areas. Persons unable to undertake the exercise will be demarcated as having skeletal fluorosis.

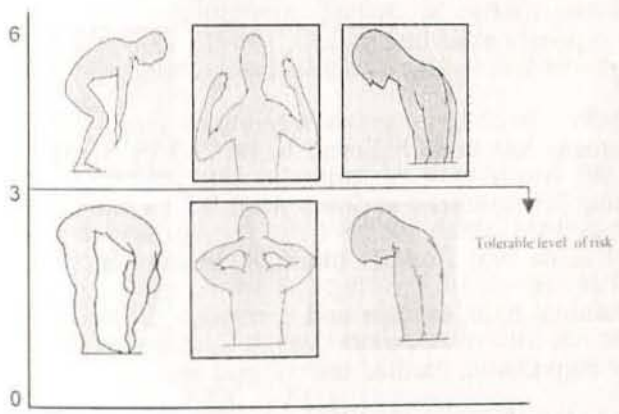

Figure 2: Identification of skeletal fluorosis and tolerable level of risk

3.4.2.3 Non- Skeletal Fluorosis

Adverse effects of fluoride are mainly dose-related, and vary with different formulations. Complaints of abdominal pain, constipation, intermittent diarrhoea, a bloated feeling, loss of appetite, a feeling of nausea and mouth sores were reported under the heading of

gastrointestinal disturbances (Susheela *et al.*, 1993). Sashi and Bhardwaj (2008) in their assessment of non-skeletal fluorosis in endemic areas found muscle weakness (38%), loss of appetite (44%), feelings of nausea (31%), abdominal pain (24%), polydypsia (27%) and polyuria (29%). Enteric-coated preparations and slow release fluoride cause fewer gastrointestinal side effects (Riggs *et al.*, 1990). Earlier studies have indicated that the incidence and severity of chronic fluoride intoxication are greatly influenced by socioeconomic status, climatic and nutritional status (Krishnamachari & Krishnamswamy 1973; Bharati *et al.*, 2005).

3.4.3 Acute toxicity

The toxicity of fluoride depends on the type of compound ingested. Generally, the more soluble salts of inorganic fluorides, such as sodium fluoride, are more toxic than those that are either weakly soluble or insoluble (WHO, 1984). Readily soluble fluoride compounds release free fluoride ions on dissolution, while fluoride compounds that are insoluble or poorly soluble donot (Liebman and Ponikvar, 2005). Fluoride from the former group, that includes sodium fluoride (NaF), hydrogen fluoride (HF), fluorosilicic acid (H_2SiF_6) and sodium monofluorophosphate (Na_2PO_3F), is easily and almost completely absorbed, while fluoride from substances in the latter group, that include calcium fluoride (CaF_2), magnesium fluoride (MgF_2) and aluminium fluoride (AlF_3), is poorly absorbed (WHO, 1984).

The acute toxic dose of fluoride in humans has been believed to be 2–5 or 8 mg/kg body weight; however, acute fluoride poisoning has occurred at doses from 0.1 to 0.8 mg/kg body weight (Akinawa, 1997). Symptoms of acute oral fluoride intoxication may include severe nausea, vomiting, hypersalivation, abdominal pain, cramps and diarrhoea; in severe or fatal cases, these symptoms are followed by convulsion, cardiac arrhythmia and coma (Akinawa, 1997). A reasonable, estimated 'certainly lethal dose' of sodium fluoride for the average 70 kg adult has been estimated to range between 5 and 10 g, corresponding to 32–65 mg fluoride/kg body weight (Whitford, 1987).

3.4.4 Fluoride intake, caries and fluorosis

Due to ubiquitous exposure to fluoride sources other than drinking water, it is difficult to draw firm conclusions regarding the independent effects of fluoride in drinking water on dental caries and its prevention. Both deficiency and excess of fluoride is associated with human health (WHO, 1984). There is a narrow range between intakes which are beneficial and those which begin to be detrimental (IPCS, 2002). It has been estimated that moderate dental fluorosis occurs in 1–2% of the population exposed to fluoride at 1 mg/L in drinking water and in about 10% of the population at 2 mg/L; moderate/severe fluorosis occurs in variable percentages ranging up to 33% of the population exposed to fluoride at 2.4–4.1 mg/L (Kaminsky 1990). According to Dean *et al.* (1942), at a fluoride concentration of 1 mg/L about 20 per cent of children have evidence of dental fluorosis but this fluorosis is of a mild degree and would not be cosmetically obvious to the children or their parents (Whitford, 1997). Thus the evidence suggested that, at least for fluoride naturally present in water, the optimal level of fluoride for a temperate climate was around 1 mg/L; this concentration was associated with a substantial resistance to tooth decay but with only a small and cosmetically insignificant increase in the prevalence of dental fluorosis (Dean, 1942).

To utilize the maximum cariostatic effect, fluoride had to become incorporated into dental enamel during development. Hence, a certain prevalence and severity of fluorosis in a population was inevitable if the prevalence and severity of caries among children was to be minimized. Dental fluorosis was regarded as an unfortunate side effect of fluoride's caries-protective benefits, and attempts to play down the possible toxic effect of fluoride on developing dental enamel often led the dental profession to present dental fluorosis as merely a cosmetic problem (Aoba & Fejerskov 2002). But as fluoride concentration increased further (up to 2.6 mg/L) dental decay continues to fall, but only slightly (Dean, 1942) while dental fluorosis increases (NRC, 1993).

NRC (2006) suggested that mild and moderate dental fluorosis is cosmetic; however, they felt that severe fluorosis had an adverse health impact because it damages the enamel and reduces its efficacy in protecting the teeth from decay. Dental decay is the destruction of the outer coating of the tooth (enamel) through the action of bacteria in the dental plaque. If decay is

untreated it spreads into the inner portion of the tooth causing a toothache and sometimes infection (NRC, 2006).

As noted by NRC (2006), the weight of evidence indicates that the threshold for severe dental fluorosis occurs at a water fluoride level of about 2 mg/L. Calcium deficiency, co-exposure to certain minerals, malnutrition, respiratory, and various pathological conditions affecting urinary output and kidney function; may contribute to increases in the prevalence and severity of dental fluorosis and/or produce dental abnormalities that are indistinguishable from dental fluorosis. Of greater concern to the NRC (2006), however, is the possibility that those individuals exposed to fluoride levels above 2 mg/L and suffering from severe fluorosis might be at greater risk of developing caries due to the fluoride-induced pitting of the enamel which would allow food plaque to become entrapped in enamel defects and thereby induce decay. Evidence of an increase in decay rates in this segment of exposed populations would support the supposition that severe fluorosis is not merely an undesirable cosmetic effect, but can also have adverse consequences with the potential to impact health (US EPA, 2010).

The relationship between caries and fluoride exposure displays the U-shaped dose-response (Figure 3) that characterizes many nutrients where there are adverse effects with intakes that are below those that confer a benefit and adverse effects with intakes that are greater than those with benefit. The base of the U identifies the dose range that defines intakes providing nutritional benefit without risk of adversity for healthy populations (US EPA, 2010). NRC (2006) concluded that in 11 of the 14 available contrasts, the measure of caries frequency was higher with severe fluorosis than with mild to moderate fluorosis.

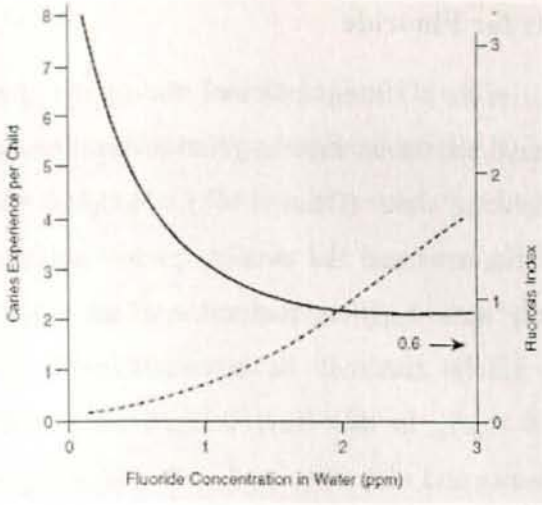

Figure 3: Dose- response of fluoride

(Relationships among caries experience (solid line), dental fluorosis index (dashed line), and the fluoride concentration of drinking water. A fluorosis index value of 0.6 was judged to represent the threshold for a problem of public health significance (Dean, 1942)).

The base of the U-shaped fluoride-caries relationship seems to occur at a drinking water concentration around 2 mg/L (US EPA, 2010). Nevertheless, the weight of evidence does support the conclusion of the NRC (2006) that, under some circumstances, severe fluorosis may be associated with an increased prevalence of caries. The US EPA (2010) finding on the caries association is also consistent with NRC (2006) and concluded that the "available evidence is mixed but generally supportive".

A study conducted in Ethiopian children by Wondwossen *et al.*, (2004a) was also consistent with NRC (2006) findings; that second molars were the teeth type most frequently affected by dental fluorosis and there is direct relationship between the severities of dental fluorosis with that of dental caries. In general in many of the non-US studies evaluated by the NRC (i.e., Ethiopia, Gaza Strip, Turkey), caries prevalence was lowest in groups showing no fluorosis, and there was a progressive increase in caries with increasing severity of dental fluorosis. Some of the factors which might account for differences in the fluorosis/caries relationship between the US and other countries include differences in dental care, dental hygiene practices, dietary habits (i.e., consumption of sugars), and nutrient intakes (i.e., calcium balance) (US EPA, 2010).

3.5 Indicators for Estimating Requirements for Fluoride

The effectiveness of fluoride against dental caries is a strong indicator for applying an adequate intake (AI). Epidemiological studies have shown an inverse relationship between dental caries and concentration of fluoride in drinking water (Dean, 1942). Hodge (1950) plotted the average community index of dental fluorosis and the average caries incidence against the concentration of fluoride in community water supplies. Reduction of the average occurrence of dental caries per child was nearly maximal in communities having concentrations of fluoride in water close to 1.0 mg/L. In this way, 1 mg/L became the 'optimal' concentration for fluoride in drinking water and was associated with a high degree of protection against dental caries and low prevalence of the milder forms of dental fluorosis.

Reports published 40–50 years ago suggested that the long-term ingestion of fluoride in amounts slightly above the optimum for caries prevention improved the quality of the human skeleton and that the risk of osteoporosis might thereby be reduced (WHO, 2002). Since the 1960s, fluoride at high dose levels has been used to treat age-dependent osteoporosis. While it appears that such treatment increases trabecular bone density, it apparently has no similar beneficial effect on cortical bone. Although such treatment may protect against vertebral fractures, data on other fractures, including femoral bone, are highly controversial (Riggs *et al.*, 1990; WHO, 2002; OSC, 2003)

Assessment of the efficacy of fluoride therapies for osteoporosis is beyond the scope of this paper. Nevertheless, it is important to note that fluoride is currently not recommended for its treatment (OSC, 2003). While slow release fluoride is reportedly beneficial, its long-term benefit is unknown (Beers & Berkow, 1999). Other data that suggest beneficial effects of fluoride other than prevention of dental caries, or increase bone mineral content, are insufficiently firm to provide the basis for estimating an AI (SCSEDRI, 1997). Several studies have shown that the fluoride balance can be negative (SCSEDRI, 1997). This occurs when the chronic intake is reduced to the extent that concentrations of fluoride in plasma fall and mobilization of fluoride from calcified tissues proceeds. In the present state of knowledge, therefore, negative fluoride balance cannot be used for establishing an AI of fluoride (WHO/FAO/IAEA) (1996).

3.5.1 Adequate Intake (AI) of Fluoride

The estimated average requirement (EAR) is defined as the nutritional intake value that is estimated to meet the requirement defined by a specified indicator of adequacy in 50% of the individuals in a particular life stage and gender group. The recommended dietary allowances (RDA), on the other hand, is the average daily dietary intake level that is sufficient to meet the nutrient requirements of nearly all (97–98%) individuals in a particular life stage and gender group SCSEDRI, 1997).

The RDA applies to individuals, not to groups. The EAR serves as the basis for setting the RDA (SCSEDRI, 1997). If adequate scientific documentation for calculating an EAR is not available, as is the case of fluoride, the AI is set instead of an RDA (SCSEDRI, 1997). The AI is based on observed or experimentally determined estimates of average nutrient intake by a group (or groups) of healthy people. Burt (1992) reviewed the history of development of the 'optimum' intake of fluoride in children. In the same report he estimated the 'average daily diet' to be in the range of 1.0–1.5 mg of fluoride. McClure (1943) suggested that 0.05 mg fluoride/day/kg body weight for children aged 1–12 years was optimum intake.

3.5.2 Enough or Too Much Fluoride

The beneficial effects of small amounts of fluoride have been established in the prevention of dental caries and thus constitute a strong indicator for an appropriate intake of fluoride, especially in children. On the other hand, excessive intake of fluoride during enamel maturation before tooth eruption, from birth to 7–8 years of age when enamel formation is complete, can lead to the development of dental fluorosis. It is the total ingested and bioavailable fluoride that is important when considering the prevention of dental caries together with the occurrence of dental fluorosis.

The Standing Committee on the Scientific Evaluation of Dietary Reference Intakes (SCSEDRI, 1997) in 1997 defined the AI for fluoride based on the extensively documented relationship between caries experience and both concentration of fluoride in water and

fluoride intake. The AI for fluoride from all sources is set at 0.05 mg/day/kg body weight. This intake range is recommended for ages above 6 months because it confers a high level of protection against dental caries and is associated with no known unwanted health effects. Despite this, thresholds of 0.05–0.07 mg/day/kg body weight of fluoride have been suggested for the appearance of dental fluorosis (Fejerskov *et al.*, 1987; Baelum *et al.*, 1987; Burt 1992).

The wide variations in fluoride intake reported in the literature make its accurate estimation difficult. High intake of fluoride in non-fluoridated areas is ascribed to the use of fluoride supplements and in fluoridated areas to fluoride added into water. Both intakes exceed the lower threshold and are close to the upper threshold of 0.07 mg/day/kg body weight for the appearance of dental fluorosis.

The results from different studies used for the determination of adequate intake are difficult to compare because

i. sample pre-treatment methods were used that do not necessarily ensure complete release of fluoride from the sample matrix,
ii. adequate information as to how the studies were conducted is not always provided and
iii. advances in analytical techniques.

In order to make reliable comparisons it is therefore suggested that, in future studies, decomposition methods that are known to release all the fluoride should be used. Use of certified reference materials (CRM) as part of the quality assurance system should be mandatory. In addition, sufficient information to enable proper comparison of data from different studies must be provided (EFSA, 2005).

Based on the available literature and the current recommendations on fluoride intake, it is hard to say whether the current AI is appropriate; too low or too high. The margin between the beneficial and deleterious effects of fluoride appears to be narrow. More accurate information on background amounts of fluoride intake, especially in children, from food, water, beverages, fluoride supplements and dentifrices is a pre-requisite for making correct decisions on the use of fluoride products (SCSEDRI, 1997).

3.6 Sources of Fluoride and its contribution to body burden

Unlike some of the other halogens, the majority of fluoride in the Earth's surface is derived from rock minerals, whereas other sources such as air, seawater and anthropogenic activities constitute a relatively small proportion. In groundwater, for example concentrations vary with the type of rock and the water that flows through it (Fordyce *et al.*, 2001). In general fluoride is found in soil, water, food and air. The fluoride content in soil normally ranges from 200 to 300 mg/L. Virtually all foodstuffs contain at least trace amounts of fluoride (WHO, 2002). Fluoride enters in human food-and-beverage chain in increasing amounts through the consumption of tea, wheat, spinach, cabbage, carrots and other food items (Susheela, 2003). Principally fluoride is ingested into human body via water, food and beverages. The amount of fluoride that a person breathes in a day is usually less than 1.0 μg/m3. Air and soil are typically responsible for only a small fraction of total fluoride exposure (Fordyce *et al.*, 2001).

The major causes for the distribution, transportation and transformation of fluoride in the environment are: weathering and dissolution of minerals in water bodies, emissions from volcanoes, marine aerosols, coal combustion and process waters and waste from various industrial processes; including steel manufacturing, primary aluminium, copper and nickel production, phosphate ore processing, phosphate fertilizer production and use, glass, brick and ceramic manufacturing, and glue and adhesive production (Toyoda and Taira, 2000). The major sources of fluoride for human exposure are water, food, beverage, tooth paste, fluoride supplements and infant formulas (EFSA, 2008).

3.6.1 Fluoride in the Environment

Fluorides are released into the environment naturally through the weathering and dissolution of minerals, in emissions from volcanoes, underground rock (Gizaw, 1996) and in marine aerosols (Symonds *et al.*, (1988) ATSDR, 2003). The anthropogenic sources of fluoride and fluoride-containing emissions include release into the environment through coal combustion and in process waters and waste from various industrial processes, including steel manufacture, primary aluminium, copper and nickel production, phosphate ore processing,

phosphate fertilizer production and use, glass, brick and ceramic manufacturing, and glue and adhesive production. The use of fluoride containing pesticides and the controlled fluoridation of drinking water supplies also contribute to the release of fluoride from anthropogenic sources (WHO, 2002).

3.6.1.1 Fluoride in the lithosphere

Fluorine, combined chemically in the form of fluorides, constitutes 0.065% of the earth's crust, being the 13th element in abundance and occurring more widely than chlorine and 5–10 times more abundantly than zinc or copper. In rock and soil, fluoride occurs in a wide variety of minerals, including fluorspar (CaF_2), cryolite (Na_3AlF_6), apatite ($Ca_5(PO_4)_3(OH,F,Cl)$ and in groups of minerals such as mica, hornblende and a number of pegmatites such as topaz and tourmaline. Despite its obvious abundance, however, most of it is, under normal conditions, firmly bound to minerals and other compounds and therefore not available to plants and animals in its usual biological form of fluoride ion (O'Donnell, 1973).

Fluoride is a natural component of most types of soil, in which it is mainly bound in complexes and not readily leached. The major source of free fluoride ion in soil is the weathering and dissolution of fluoride rich rock that depends on the natural solubility of the fluoride compound in question, pH, and the presence of other minerals and compounds and of water. The major parameters that control fluoride fixation in soil through adsorption, anion exchange, precipitation, formation of mixed solids and complexes are aluminium, calcium, iron, pH, organic matter and clay (Tripathy *et al.*, 2005). Larsen and Widdowson (1971) estimated the average fluoride concentration in soil to be about 300 mg/kg. The amount in organic soils is usually lower than in mineral soils.

3.6.1.2 Fluoride in air

Airborne fluoride exists in gaseous and particulate forms emitted from both natural and anthropogenic sources (WHO, 2002). Industrial production of phosphate fertilizers, coal ash from the burning of coal and volcanic activity are the main sources (NRC, 1993; Murray, 1986). The gaseous fluorides include hydrogen fluoride (HF), carbon tetrafluoride (CF_4), hexafluoroethane (C_2F_6) and Silicon tetrafluoride (SiF_4), while particulate fluorides include

cryolite (Na_3AlF_6), chiolite ($Na_5Al_3F_{14}$), calcium fluoride (CaF_2), aluminium fluoride (AlF_3) and sodium fluoride (NaF) (WHO, 2002). The distribution and deposition of airborne fluoride are dependent upon emission strength, meteorological conditions, topography, particle size and chemical reactivity. (Low and Bloom, 1988). According to John *et al.*, (2009) the amount of fluoride inhaled per day from air is very low

Volcanoes and photolysis of anthropogenic halocarbons in the stratosphere contribute significant amount of gaseous HF into troposphere and stratosphere (Symonds *et al.*, 1988). Another important natural source of fluoride is sea salt released from the oceans in the form of marine aerosols. Anthropogenic sources of fluoride include fossil fuel combustion and industrial waste. Hydrogen fluoride is water soluble and emissions are readily controlled by acid gas scrubbers (Cadle 1980). Apparently only limited data are available concerning total annual emissions of HF from industrial operations; however, there is evidence that emissions of fluorides have been declining (Cadle 1980; ATSDR, 2003).

Fluorine-containing gases do not disperse so rapidly in the atmosphere as other gases. Smoke is often carried over great distances and can cause considerable damage when it finally descends. Molecular fluorine and hydrogen fluoride are largely responsible for the plant damage caused by stack gases (Tressaud, 2006). Fluoro-organics have effects on plants and organisms (Davison & Weinstein, 2006). Hydrogen fluoride is reportedly 1000 times as harmful as sulfur dioxide and there can be no doubt that the harmful effect of the latter on plants is greatly exacerbated by traces of fluorine (Bergmann, 1971).

3.6.1.3 Fluoride in the phosphate ore

Fluorine, hydrogen fluoride, and other volatile fluorides are produced in the manufacture of aluminum. Hydrogen fluoride is a by-product in the conversion of fluorapatite (rock phosphate) to phosphoric acid, superphosphate fertilizers, and other phosphorus products. The wet process for the production of phosphoric acid involves the reaction of fluorapatite, $Ca_5F(PO_4)_3$, with sulfuric acid:

$$Ca_5F(PO_4)_3 + 5H_2SO_4 + 10H_2O \longrightarrow CaSO_4.2H_2O + HF + 3H_3PO_4$$

It is necessary to recover most of the by-product fluorine from rock phosphate processing to avoid severe pollution problems. Recovery as fluorosilicic acid, H2SiF6, is normally practiced. Hydrogen fluoride gas is a dangerous substance that is so corrosive it even reacts with glass. It is irritating to body tissues, and the respiratory tract is very sensitive to it. Brief exposure to HF vapors at the part-per-thousand level may be fatal. The acute toxicity of F_2 is even higher than that of HF. Chronic exposure to high levels of fluorides cause fluorosis, the symptoms of which include mottled teeth and pathological bone conditions (Mahnan, 2003).

Plants are particularly susceptible to the effects of gaseous fluorides. Fluorides from the atmosphere appear to enter the leaf tissue through the stomata. Fluoride is a cumulative poison in plants, and exposure of sensitive plants to even very low levels of fluorides for prolonged periods results in damage. Characteristic symptoms of fluoride poisoning are chlorosis (fading of green color due to conditions other than the absence of light), edge burn, and tip burn. Conifers (such as pine trees) afflicted with fluoride poisoning may have reddish-brown, necrotic needle tips. The sensitivity of some conifers to fluoride poisoning is illustrated by the fact that fluorine produced by aluminum plants in Norway has destroyed forests of *Pinus sylvestris* up to 8 miles distant; trees were damaged at distances as great as 20 miles from the plant (Mahnan, 2003).

Silicon tetrafluoride gas, SiF_4, is another gaseous fluoride pollutant produced during some steel and metal smelting operations that employ CaF_2, fluorspar. Fluorspar reacts with silicon dioxide (sand), releasing SiF4 gas:

$$2CaF_2 + 3SiO_2 \longrightarrow CaSiO_3 + SiF_4$$

Another gaseous fluorine compound, sulfur hexafluoride, SF6, occurs in the atmosphere at levels of about 0.3 parts per trillion. It is extremely unreactive with an atmospheric lifetime estimated at 3200 years, and is used as an atmospheric tracer.

Silicon hexzfluoride does not absorb ultraviolet light in either the troposphere or stratosphere, and is probably destroyed above 60 km by reactions beginning with its capture of free electrons. Current atmospheric levels of SF6 are significantly higher than the estimated background level of 0.04 ppt in 1953 when commercial production of it began. The compound is very useful in specialized applications including gas insulated electrical equipment and inert blanketing/degassing of molten aluminum and magnesium. Increasing uses of sulfur hexafluoride have caused concern because it is the most powerful greenhouse gas known, with a global warming potential (per molecule added to the atmosphere) about 23,900 times that of carbon dioxide (Mahanan, 2003).

3.6.1.4 Fluoride from Volcanic Sublimates

A number of mineral substances are gaseous at the magmatic temperatures of volcanoes and are mobilized with volcanic gases. These kinds of substances condense near the mouths of volcanic fumaroles and are called sublimates. Fluoride and chloride sublimates are sources of gaseous HF and HCl formed by their reactions at high temperatures with water, such as the following:

$$2H2O + SiF4 \longrightarrow 4HF + SiO2$$

3.6.1.5 Fluoride in natural waters

Natural waters contain fluorides in varying concentrations, which depend mainly on factors such as availability and solubility of minerals containing fluoride, porosity of the rock and soils through which the water passes, residence time, temperature, pH and the presence of other elements (Ayoob and Gupta, 2006; Gizaw 1996). Concentrations of fluoride in water also vary according to the natural sources of emission and to anthropogenic discharges that lead to increased levels of fluoride in the environment (WHO, 1984).

Fluoride levels of groundwater are higher than in surface water because they are more influenced by the rocks through which they pass (ASTDR, 2003). Waters with high concentrations of fluoride are usually found at the foot of high mountains and in areas with

geological deposits of marine origin. Typical examples are the geographical belt that extends from Syrian to the African Rift Valley (Figure 3). Another belt is that stretching from Turkey through Iraq, Iran and Afghanistan, to India, northern Thailand and China (WHO 1994).

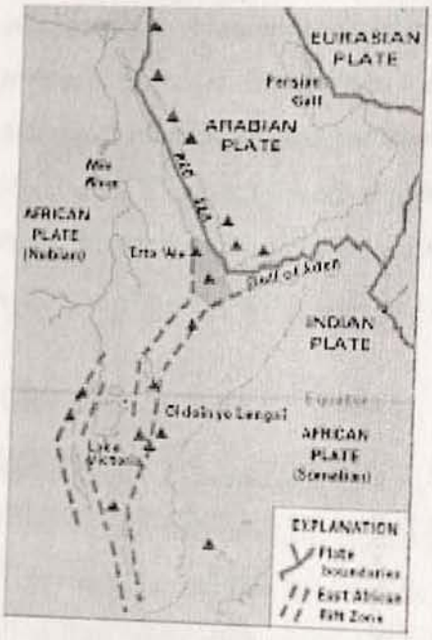

Figure 4: African rift System

Most of the water normally available to humans is involved in the hydrological cycle, which means that it originates in the sea. Fluoride is usually transported through the water cycle bound by an excess of aluminium (Ares, 1990). Concentrations of fluoride in seawater are higher than in fresh water (WHO 1994; ATSDR, 2003). The amount of fluoride in water in areas with high naturally occurring fluoride is usually above 1 mg/L, reaching up to 50 mg/L in springs and geysers. The highest value ever found in water was recorded in Lake Nakuru in the Rift Valley in Kenya, namely 2800 mg/L (WHO, 2002).

Fluoride in water derives mainly from dissolution of natural minerals in the rocks and soils with which water interacts. High fluoride concentrations are also often found in arid climatic conditions. Here, groundwater flow is slow and reaction times between water and rocks are therefore increased (Ayoob and Gupta, 2006). The general nature of the geology is not always an indicator of the concentrations of fluoride in groundwater. There are significant variations

in the distribution of rocks with readily leaching fluoride. It has been observed that, even within one community, different wells often show widely divergent concentrations of fluoride, apparently as a result of differences in the local hydrogeological conditions (WHO, 1994).

3.6.2 Water fluoridation

The major source of dietary fluoride in many countries is drinking water either fluoridated or naturally contaminated. Controlled addition of fluoride to water is used by communities as a public health measure to adjust fluoride concentration in drinking water to an optimal level of 0.7-1.2 ppm. This concentration range has been found to decrease the incidence of dental caries while minimizing the risk of dental fluorosis and other adverse effects. Approximately 74% of the US population receives water with sufficient fluoride for the prevention of dental caries (NCCDPHP, 2010; WHO, 2008).

Fluoridation of water in New Zealand is largely accepted, except in two major cities that do not adjust the fluoride level of their water supply. Referendum is becoming the norm for determining public opinion on whether to fluoridate or not. In many cities in the western world, drinking water is fluoridated to help prevent people's teeth from decaying. Fluorine achieves this by replacing hydroxyapatite ($Ca_5(PO_4)_3OH$) with fluoroapatite ($Ca_5(PO_4)_3F$). Fluoroapatite is more resistant to acid attack and thus teeth which contain even a small proportion of fluoroapatite are less likely to decay. The relevant reactions are as follows:

Tooth decay: $Ca_5(PO_4)_3OH(s) + 4H_3O^+(aq) \rightarrow 5Ca^{2+}(aq) + 3HPO_4^{2-}(aq) + 5H_2O(l)$

Fluoridation: $Ca_5(PO_4)_3OH(s) + F^-(aq) \rightarrow Ca_5(PO_4)_3F(s) + OH^-(aq)$

Three chemicals namely sodium fluoride, sodium fluorosilicate and hydrofluorosilicic acid (HFA) are in common use for the purpose of fluoridation.

3.6.3 Food and Beverages

Drinking-water is typically the largest single contributor to daily fluoride intake (Murray, 1986). However, fluoride intake from other sources is also considerable, especially from food (Toyoda and Taira, 2000; Malde *et al.*, 1997). Han and his colleagues (1995) found that food is the main causative factor for fluorosis in areas with low fluoride concentration in the drinking water. Although the fluoride content of most foods is low (less than 0.05 mg/100 grams or 0.5 ppm), high fluoride concentration is often found in food ingredients rich in minerals and trace elements. Teff flour, for instance, contain high level of fluoride, iron, zinc and calcium (FAO, 1987; Maage *et al.*, 1996). Rich sources of fluoride include tea, which concentrates fluoride in its leaves, and marine fish that are consumed with their bones (e.g., sardines). Foods made with mechanically separated (boned) chicken, such as canned meats, hot dogs, and infant foods, also add fluoride to the diet (Fein *et al.*, 2001). In addition, certain fruit juices, particularly grape juices, often have relatively high fluoride concentrations (Kiritsy *et al., 1996).* According to Weinsten (1977), the highest fluoride concentrations are found in the leaves and roots of plants.

Except for tea, most vegetables grown in low-fluoride areas have fluoride content less than 1mg/kg (Singer et al., 1986). Tea plants are found having high fluoride uptake and 97% of it gets accumulated in leaves (Shu *et al.*, 2003). The fluoride content of tea leaves is about 1,000 times the soluble fluoride content of soil and 2 to 7 times the total fluoride content in soil (Fung *et al.*, 1999). Two-three cups of tea contain approximately 0.4–0.8 mg fluoride (IPCS, 1984). Zerabruk and his colleagues (2010) in their assessment of tea leaves brewed in low fluoride containing water found enormous amount of fluoride level in local market in Ethiopia.

With relatively high fish consumption in a mixed diet, the fluoride intake from fish alone would seldom exceed 0.2 mg F- per day (Murray, 1986). Milk typically contains low levels of fluoride, e.g. 0.02 mg/L in human breast milk and 0.02–0.05 mg/L in cow's milk (Murray, 1986). Thus milk is usually responsible for only a small fraction of total fluoride exposure. Vegetables and fruits normally have low levels of fluoride (e.g. 0.1–0.4 mg/kg) and thus typically contribute little to exposure. However, higher levels of fluoride have been found in

barley and rice (example about 2 mg/kg). The levels of fluoride in meat (0.2–1.0 mg/kg) and fish (2–5 mg/ kg) are also relatively low (Murray, 1986). However, according to the weight of evidence obtained from WHO (2004) for children, total fluoride intake via food and water is decreased because of lower consumption. An adult male residing in a community with fluoridated water has an intake range from 1-3 mg/day. Intake is less than 1 mg/day in non-fluoridated areas (Nielsen, 1999). In fluoride contaminated areas estimation of total dietary fluoride intake in individuals may be based either on analysis of raw food ingredients (Malde *et al.*, 1997) or analysis of selected prepared food dishes (Zohouri and Rugg-Gunn, 2000).

3.6.4 Dental Products: Toothpaste

A number of products administered to, or used by children to reduce dental decay contain fluoride. This includes toothpaste (1–1.5 mg/ g fluoride) (Murray, 1986). Fluoridated toothpastes are believed very effective in preventing dental caries but also add considerably to fluoride intake of children, especially young children who are more likely to swallow toothpaste. Researchers estimate that children under 6 years of age may ingest an average of 0.3 mg of fluoride from toothpaste with each brushing. Children under the age of 6 years who ingest more than two or three times the recommended fluoride intake are at increased risk of a white speckling or mottling of the permanent teeth, known as dental fluorosis. A major source of excess fluoride intake in this age group comes from swallowing fluoride-containing toothpaste. To prevent dental fluorosis while providing optimum protection from tooth decay, it is recommended that parents supervise children under 6 years of age while brushing with fluoridated toothpaste (ADA CSA, 2014). Interestingly, it has been suggested that the management of the fluorosis risk in young children who ingest fluoridated toothpaste could include the use of toothpaste formulation that reduces gastrointestinal absorption and bioavailability of fluoride (Falco et al., 2013).

3.6.5 Infant formulas

While consumption of fluoride from water presents very little risk of adverse effects in adults except in extreme circumstances, consumption of relatively large amounts of water mixed with formula concentrates appears to increase the risk for the development of dental fluorosis in infants (Marshould *et al.*, 2004; Pendrys, 2000; Levy *et al.*, 2010). One study found that,

on average, at least half of all fluoride ingested by infants 6 months and younger was from water mixed with formula concentrates (Levy *et al.*, 1995). The study of 49 commercially available infant formulas in the Chicago area showed that milk-based ready-to-feed, liquid concentrate and powdered formulas (reconstituted with deionized water) had mean fluoride concentrations of 0.15 ppm, 0.27 ppm, and 0.12 ppm, respectively. Fluoride-free or low-fluoride water labeled as "deionized," "purified," "demineralized," "distilled," or "produced through reverse-osmosis" can be used in order to minimize the risk for mild fluorosis (Palmer and Gilbert, 2012).

American Dental Association Fluoride Supplement Schedule (ADA CSA, 2014)

Age	**Fluoride ion level in drinking water (ppm)***		
	< 0.3 ppm	**0.3-0.6 ppm**	**> 0.6 ppm**
Birth - 6 months	None	None	None
6 months - 3 years	0.25 mg/day**	None	None
3 years - 6 years	0.50 mg/day	0.25 mg/day	None
6 years -16 years	1.0 mg/day	0.50 mg/day	None

* 1.0 part per million (ppm) = 1 milligram/liter (mg/L) ** 2.2 mg sodium fluoride contains 1 mg fluoride ion.

3.6.6 Fluoride supplements

Fluoride supplements by prescription are available for infants and children up to 16 years of age living in areas with suboptimal water fluoridation for the purpose of bringing their intake to approximately 1 mg/day (Alvarez *et al.*, 2009). The American Dental Association Council on Scientific Affairs recommends the prescription of fluoride supplements only to children at high risk of developing dental caries (Rozier *et al.*, 2010). The supplemental fluoride dosage schedule in the table below was recommended by the American Dental Association, the American Academy of Pediatric Dentistry, and the American Academy of Pediatrics (Rozier *et al.*, 2010; CDC, 2001). It requires knowledge of the fluoride concentration of local drinking water, as well as other possible sources of fluoride intake.

3.6.7 Salt fluoridation

Fluoridation of salt has been implemented in several countries worldwide as an alternative to water fluoridation to promote the ingestion of fluoride and improve oral care. Since the fluoridation of water is extensively practiced in the US, fluoride is not added to salt. Epidemiological studies have shown that the incidence of teeth with caries dramatically decreased in the regions where salt fluoridation programs were developed. While concerns around hypertension and the monitoring of population intakes should be addressed, no adverse health effects linked to the fluoridation of salt have been reported (Pollick, 2013). According to the World Health Organization (WHO), salt fluoridation and, to a lesser extent, milk fluoridation are affordable alternatives to improve oral hygiene in areas where access to oral health services is limited and fluoridation of public water is not feasible (Marthaler and Petersen, 2005)

3.7 Pharmacokinetics of fluoride ions

3.7.1 Oral uptake

In humans and animals, ingested fluoride occurs as hydrogen fluoride (HF) in the acidic environment of the stomach and is effectively absorbed from the gastrointestinal tract, although there is no proved absorption from the oral cavity. Peak plasma levels are typically seen within 30–60 minutes after ingestion. Highly soluble fluoride compounds, such as NaF present in tablets, aqueous solutions and toothpaste are almost completely absorbed, whereas compounds with lower solubility, such as CaF2, MgF2, and AlF3, are less well absorbed. Ingestion of fluoride with milk or a diet high in calcium will decrease fluoride absorption.

3.7.2 Dermal absorption

No experimental data on the extent of dermal absorption of fluoride from dilute aqueous solutions are available. As fluoride is an ion it is expected to have low membrane permeability and limited absorption through the skin from dilute aqueous solutions at near neutral pH (such as water used for bathing and showering). This exposure pathway is unlikely to contribute to the fluoride body burden.

3.7.3 Inhalation

No systematic experimental data on the absorption of fluoride after inhalation are available. A few older occupational studies have shown uptake of fluoride in heavily exposed workers from fluoride-containing dusts, but it is unlikely that inhalation exposure will contribute significantly to the body burden of fluoride in the general population.

3.8 Absorption, Metabolism and Excretion of Fluoride

3.8.1 Absorption

The metabolism of fluoride and the effects of normal and abnormal intake of fluoride on body processes have been investigated extensively. The concentration of fluoride ion is generally considered to be more significant than that of total fluorine in assessing the effects of excessive fluorine intake on normal metabolism as it do not exist free in nature. However, if fluoride binds to the enzyme, substrate, or the co-factor of a given enzymatic reaction, the bound fluoride will affect the overall process. Although the importance of ionic fluoride is generally recognized, it is total fluorine rather than ionic fluoride concentrations that have been reported in most publications. In humans, the predominant route of absorption of fluoride is via the gastrointestinal tract. Airborne fluoride may also be inhaled. Dermal absorption is negligible except in cases of hydrofluoric acid burns (WHO 1994).

Absorption of fluoride varies from 100% on a fasting stomach, to 60% when taken with a calcium-rich breakfast. A fasting child may absorb fluoride from water more quickly than a well fed child because in an empty stomach fluoride does not form complexes with other substances (Ekstrand and Ehernebo, 1979). When ionic fluoride enters the acidic environment of the stomach lumen, it is largely converted to weak acid hydrogen fluoride (HF) with a pKa of 3.45 (WHO, 2002). The higher acidity of the stomach speeds up the process of absorption by passive diffusion from both the stomach and the small intestine, suggesting that fluoride is absorbed from the stomach as undissociated hydrogen fluoride rather than as fluoride ion (Cerklewski, 1997; Whitford & Pashley, 1984). In an acidic stomach (low pH), fluoride is converted into hydrogen fluoride (HF) and up to about 40 per cent of the ingested fluoride is

absorbed from the stomach as HF. On the other hand high stomach pH decreases gastric absorption by decreasing the concentration of HF. Fluoride not absorbed in the stomach is absorbed in the intestine and is unaffected by pH (Whitford, 1997; IPCS, 2002). Most of the fluoride that is not absorbed from the stomach will be rapidly absorbed from the small intestine. There is no convincing evidence that active transport processes are involved (WHO, 2002).

Reports indicated that soluble forms of fluoride salts have absorption efficiencies of between 80–100 % (ATSDR, 2003). It has been shown that ingestion of fluoride with a meal can increase the absorption of fluoride (Trautner and Einwag 1987) thereby increasing bioavailability, but concomitant supplementation of sodium fluoride with milk or dairy products reduced its availability by 13–50% in humans and animals (ATSDR, 2003). Approximately 90% of fluoride ingested in water is absorbed in the gastrointestinal tract compared to only 30 – 60% of fluoride in food (WHO, 1996).

3.8.2 Bioavailability: Nutrient interactions

Bioavailability may be defined as the extent to which the active moiety (drug or metabolite) enters the systemic circulation, thereby gaining access to the site of action (Beers & Berkow, 1999). A common way of studying the bioavailability of drugs / nutrient is to compare plasma concentration curves and urinary excretion data after a single oral dose, with similar data obtained after intravenous administration. By definition, when a medication is administered intravenously, its bioavailability is 100%. Fluoride compounds that occur naturally or are added to drinking water and yield fluoride ions on dissolution generally exhibit high bioavailability.

Relative to the amount of fluoride ingested, high concentrations of cations that form insoluble complexes with fluoride (e.g. calcium, magnesium and aluminium) can markedly decrease gastrointestinal fluoride absorption (Whitford, 1997; IPCS, 2002). However, the absorption of fluoride in the form of monofluorophosphate (unlike sodium fluoride) is unaffected by calcium. Also, a diet low in chloride (salt) has been found to increase fluoride retention by

reducing urinary excretion of fluoride (Cerklewski, 1997). In infants about 80 to 90 per cent of the absorbed fluoride is retained but in adults this level falls to about 60 per cent.

Bioavailability is lower in the presence of high dietary concentrations of calcium and certain other divalent or trivalent cations with which fluoride can form insoluble or poorly soluble compounds. Nutritional and clinical aspects of fluoride bioavailability have been reviewed by Cerklewski (1997).

Most studies assessing bioavailability of fluoride in humans have concentrated on comparing fluoride concentration in the plasma after intake of a substance or food, the values obtained then being compared to plasma values obtained after intravenous or oral administration of sodium fluoride (Ekstrand *et al.*, 1978; Trautner & Siebert, 1986; Trautner &Einwag, 1989; Shulman & Vallejo, 1990; Goyal *et al.*, 1998)). Studies on the bioavailability of fluoride, based on both plasma and urine values of fluoride were also reported. The methodological approach of these studies is based on the assumptions that sodium fluoride is fully absorbed (100%) on an empty stomach, no endogenous loss of fluoride occurs and urinary excretion is the major route of fluoride elimination (Ekstrand & Ehrnebo, 1979).

The bioavailability, from a fasting stomach, of fluoride from sodium fluoride tablets, as used in many caries-prevention programs, was almost 100% (Ekstrand *et al* , 1978). In studies on adults, this bioavailability was decreased to 50–79% by co-administration of milk or calcium rich products (Ekstrand & Ehrnebo, 1979). Yet in another study it was shown that this effect of milk was abolished when fluoride was taken as a part of the breakfast Trautner &Einwag, (1989). The authors suggested that formation of calcium salts and entrapment of fluoride in coagulation products of milk are important factors causing the reduction of fluoride bioavailability, and that prolonged stay of chyme after concomitant ingestion of food allows fluoride to become liberated from the bound forms and coagulation products by digestion (Trautner &Einwag, 1989).

The poor fluoride bioavailability, in the range of 4–24%, observed from food such as bone meal, fish bone meal, canned sardines and chicken bone meal was ascribed to the high content of calcium in these foods so only part of the total fluoride present in the food could be liberated during the digestion process (Trautner & Siebert, 1986). Low bioavailability of fluoride (2–32%) was reported in humans from diets commonly consumed in India (Goyal *et al.*, 1998). The data from the authors showed a wide range of bioavailability (2–79%) of fluoride from different foods in adults. Factors such as pH, the amount of ingested food, and the presence and type of minerals in ingested food had effect on bioavailability.

The bioavailability of fluoride from diet and fluoride supplements in infants was about 90% (Ekstrand *et al.*, 1994). Results suggest that the mode of administration of a given product is highly important for fluoride bioavailability. Bioavailability of fluoride is best determined under conditions that simulate the normal mode of intake of the products or food to be tested.

3.8.3 Fluoride tissue distribution

Once absorbed, fluoride is rapidly distributed throughout the body via the blood. The short term plasma half-life is normally in the range of 3 to 10 hours. Fluoride is distributed between the plasma and blood cells, with plasma levels being twice as high as blood cell levels (Ekstrand 1977a, b). Fluoride crosses the placenta and is found in mother's milk at low levels essentially equal to those in blood (WHO, 1996; IPCS, 2002). Levels of fluoride that are found in the bone vary with the part of the bone examined and with the age and sex of the individual. Bone fluoride is considered to be a reflection of long-term exposure to fluoride (IPCS, 2002). The saliva fluoride level is about 65% of the level in plasma (Ekstrand 1977b).

Plasma fluoride concentrations are not homeostatically regulated, but rise and fall according to the pattern of fluoride intake (NRC, 1993). In adults, plasma fluoride levels appear to be directly related to the daily exposure of fluoride. Mean plasma levels in individuals living in areas with a water fluoride concentration of 0.1 mg/L or less are normally 9.5 µg /L, compared to a mean plasma fluoride level of 19-28.5 µg/L in individuals living in areas with a water fluoride content of 1.0 mg/L. In addition to the level of chronic fluoride intake and recent intake, the level of plasma fluoride is influenced by the rates of bone accretion and

dissolution, and by the renal clearance rate of fluoride (Tressaud and Haufe, 2008).

Approximately 99% of the fluoride in the human body is found in bones and teeth. Fluoride is incorporated into tooth and bone by replacing the hydroxyl ion in hydroxyapatite to form fluorohydroxyapatite. The level of fluoride in bone is influenced by several factors including age, past and present fluoride intake, and the rate of bone turnover. Fluoride is not irreversibly bound to bone and is mobilized from bone through bone remodeling (NRC, 1993).

Soft tissues do not accumulate fluoride, but a higher concentration has been reported for the kidney due to the partial re-absorption. The blood-brain barrier limits the diffusion of fluoride into the central nervous system, where the fluoride level is only about 20% that of plasma. Human studies have shown that fluoride is transferred across the placenta, and there is a direct relationship between fluoride levels in maternal and cord blood. In humans, fluoride is poorly transferred from plasma to milk. The fluoride concentration in human milk is in the range of 3.8–7.6 µg/L.

3.8.3.1 Plasma fluoride

Fluoride taken in the form of sodium fluoride as a tablet or solution is absorbed rapidly. Only a few minutes after intake, there is a rise in plasma fluoride. The fluctuation in plasma fluoride concentration is dependent on the fluoride dose ingested, the dose frequency and the plasma half-life of fluoride. The half time for absorption is 30 min, so peak plasma concentration usually occurs within 30–60 min (Ekstrand *et al* , 1978; Trautner & Siebert, 1986). Absorbed fluoride is rapidly distributed by the circulation to the intracellular and extracellular fluids and is retained only in calcified tissues (Trautner &Einwag, 1989; Shulman & Vallejo, 1990; Goyal *et al*., 1998).

The sensitivity of the serum fluoride concentrations to previous intake, glomerular filtration and the intensity of bone resorption suggests that the human organism exerts no direct homeostatic control and that fluoride concentrations reflect the recent intake (Waterhouse *et al*., 1980). Plasma fluoride levels increase with age, with increasing fluoride content of bone and as a consequence of renal insufficiency (EFSA, 2005).

Fluoride is present in human and animal serum in two forms, inorganic fluoride and organic fluorine (Taves, 1968a; Guy *et al.*, 1976). The former consists of ionic and non-ionic fluoride and the latter of fluorine covalently bound within an organic molecule originating from natural or industrial sources (Teves *et al.*, 1970; Belisle, 1981). Average concentrations of fluoride in human plasma is dependent on the concentration of fluoride in drinking water (Guy *et al.*, 1976) but no relationship between concentrations of inorganic fluoride and organic fluorine in plasma.

3.8.3.2 Fluoride in bone and calcified tissue

Fluoride is rapidly distributed in plasma and deposited in bone and other calcified tissues that contain 99% of the body's fluoride; the remainder is distributed between blood and soft tissues (Kaminsky *et al.*, 1990). About 50% of daily fluoride intake is associated with the calcified tissues within 24 h and remaining 50% will be excreted in urine. This 50:50 distribution is strongly shifted to greater retention in the very young and probably towards greater excretion in the later years of life (SCSEDRI, 1997).

Fluoride is incorporated into bone by replacing the hydroxyl ion in hydroxyapatite to form fluorohydroxyapatite. Fluoride in calcified tissues is strongly but not irreversibly bound. It appears that fluoride in bone exists in two pools, one rapidly and the other slowly exchangeable (SCSEDRI, 1997). The former is located in the hydration shell of the bone crystallites, where fluoride can be exchanged isoionically or heteroionically with ions in the surrounding extracellular fluids, while mobilization from the slowly exchangeable pool results from the resorption associated with the process of bone remodelling (SCSEDRI, 1997). Typical levels in adult bone vary between 500 and 4000 mg/kg bone ash (Kaminsky *et al.*, 1990).

3.8.3.3 Fluoride in placenta and foetus

Fluoride readily crosses the placenta and is found in foetal and placental tissue. There appears to be a direct relationship between fluoride levels in maternal blood and cord blood (Shen &Taves, 1974; Gupta et *al.*, 1993; Malhotra et *al.*, 1993; Brambilla *et al.*, 1994). At relatively

low maternal blood levels, the cord blood levels were at least 60% of that of maternal blood (Gupta *et al.*, 1993; Brambilla *et al.*, 1994). Although cord fluoride levels were typically lower than maternal levels, one study found no statistical difference between maternal and newborn (1-day-old) serum fluoride levels, suggesting that cord serum fluoride levels do not reflect foetal fluoride status (Shimonovitz, *et al.*, 1995). There is also evidence, however, that the placenta can accumulate fluoride, possibly playing a regulative role that helps protect the foetus from excessive amounts of fluoride, when maternal fluoride intake is high (Shen &Taves, 1974; Gardner *et al.*, 1952).

3.8.4 Elimination/ Excretion of fluoride

Absorbed fluoride that is not deposited in calcified tissue is excreted. The primary pathway for fluoride excretion is via the kidneys and urine; to a lesser extent, fluoride is also excreted in the faeces, sweat and saliva.

3.8.4.1 Excretion via the kidneys: urine

The major route of fluoride excretion is via the kidney and urine; 40–60% of the daily intake is excreted in the urine with an elimination half-life of about 5 h (Ekstrand & Ehrnebo, 1983; WHO, 2002). The fluoride ion is filtered from the plasma by the glomerulus and then partially reabsorbed; there is no tubular secretion of fluoride. Renal clearance rates of fluoride in humans average at 50 mL/minute (Ekstrand, 1977b). Fluoride excretion is influenced by a number of factors, including glomerular filtration rate, urinary flow and urinary pH. The excretion of fluoride in urine is reduced in individuals with impaired renal function. Urine fluoride excretion is 0.79 mg/day in humans with normal renal function, 0.53 mg/day in those with questionable and 0.27 mg/day in those with impaired renal function (Singer & Ophaug, 1982). There are no apparent age related differences in renal clearance rates (adjusted for body weight or surface area) between children and adults. However, in older adults (more than 65 years of age), a significant decline in renal clearance of fluoride has been reported; consistent with the age-related decline in glomerular filtration rates (Ekstrand, 1977b).

3.8.4.2 Excretion via faeces, saliva and sweat

Fluoride that is not aborbed will be excreted through faeces and ranges from 10–25% of the daily intake of fluoride (WHO, 1994). It has been estimated that 1% or less of an ingested dose is excreted in saliva (Oliveby, *et al.*, 1989) because saliva is swallowed, this amount does not enter mass balance calculations. In contrast to the situation for most electrolytes, the fluoride concentration in saliva appears to be independent of salivary flow rate (Oliveby *et al.*, 1989). Sweat provides only a minor route of fluoride excretion, even under extreme environmental conditions (WHO, 1994).

3.8.4.3 Excretion via breast milk

The fluoride content of human breast milk usually follows the natural daily fluoride intake during the first 6 months of life. This is especially important when comparing the daily fluoride intake by formula-fed and breastfed infants. Ekstrand *et al.* (1981) reported that in humans, fluoride is poorly transferred from plasma to breast milk. A wide range (2–50 mg/L) of fluoride in breast milk has been reported by different researchers (Ekstrand *et al.*, 1981; Esala *et al.*, 1982), although considerably higher levels, exceeding 200 mg/L, have also been reported (WHO/IAEA, 1989). The wide range of concentrations reported in human milk may be ascribed to analytical problems at low levels of fluoride.

A single dose of 1.5 mg sodium fluoride did not result in a significant rise in fluoride concentrations in breast milk within 3 hours (Ekstrand *et al.*, 1981). No correlation has been found between concentrations of fluoride in tap water and concentrations of fluoride in breast milk. Fluoride concentrations breast milk of women living in an area with high concentrations of naturally occurring fluoride in tap water was higher than those living in areas with low concentrations (Esala *et al.*, 1982).

3.9 Fluoride intake and dietary sources

3.9.1 Dietary Sources

Drinking water, food, beverages and dental products containing fluoride are regarded as the main contributors to oral fluoride intake in humans. In some countries, fluoride is also added to salt and milk.

3.9.1.1 Concentration of fluoride in drinking water

The natural concentration of fluoride in drinking water varies from trace amounts to toxic concentrations. Because of the low natural levels of fluoride in some water supplies and the high levels of dental caries, many authorities worldwide have permitted, or instigated, fluoridation of water supplies. The first artificial fluoridation trials started in 1945 in two towns in America – Newburgh and Grand Rapids (Smith, 1985). Commonly used compounds for water fluoridation are sodium or potassium fluoride or hexafluorosilicic acid (H_2SiF_6) and its sodium salt (Na_2SiF_6).

According to WHO (1994) recommendations, the absolute upper concentration for fluoride in drinking water is 1 mg/L. Yet even this concentration can be too high for many parts of the world due, not only to the greater consumption of water in hot climates, but also to the increasing levels of fluoride in, and increased consumption of, processed drinks and foods, the variety of dental practices and different lifestyles. WHO (1994) therefore suggested that the level of 1.0 mg/L should be seen as an absolute upper limit, even in cold climates, while 0.5 mg/L may be appropriate lower limit. The history of water fluoridation and its value in the early years of the 21st century have recently been reviewed by Mullen (2005).

Tap water is increasingly being replaced by the use of bottled water for drinking. Whereas drinking water for human consumption according to E.U. Council Directive 98/83/EC, following the advice of the Scientific Committee on Food (SCF, 1998), may not contain more than 1.5 mg/L of fluoride, bottled natural mineral waters may contain higher concentrations of fluoride. Mineral waters exceeding concentrations of fluoride 1.5 mg/L shall, in the meantime, bear on the label the words 'contains more than 1.5 mg/L of fluoride: not suitable

for regular consumption by infants and children under 7 years of age' and shall indicate the actual concentration of fluoride (EFSA, 2005).

According to the U.S. Food and Drug Administration (FDA), imported bottled water to which no fluoride has been added shall not contain fluoride in excess of 1.4 mg/L (FDA, 2000). The concentration of fluoride in bottled water packaged in the United States depends on the annual average of maximum daily air temperatures at the location where the bottled water is sold at retail (FDA, 2000).

3.9.1.2 Fluoride in Food

Virtually all foods contain at least trace amounts of fluoride. Factors that can influence the level of fluorides in food include the locality in which the food is grown, the amount of fertilizer and pesticides applied the type of processing the food receives and whether fluoridated water is used in their preparation. Some species of plants and marine organisms, however, appear to accumulate fluoride in large quantities.

Numerous studies have reported the results of determinations of ashed (total) and unashed (inorganic or free) fluoride in different food items. The U.S. Food and Drug Administration (FDA, 2000), for example, has collected market basket food collections of representative diets for the average young male 16–19 years of age, for the purpose of estimating the dietary intake of certain metals and of monitoring pesticide residues in the food chain. Food items in this program were divided into the composite food groups. The total free fluoride contents determined in the different food groups by market basket programme (Singer *et al.*,. 1980; Singer and Ophaug, 1986).

Taves (1983) reported the ranges and averages of the amounts of fluoride in individual foods. The amount of ashed fluoride, which represents total fluorine, is generally higher than the amount of unashed inorganic or free fluoride. The highest average amounts of ashed fluoride were determined in the meat food group (meat, poultry and fish), in grain and cereal products and beverages.

The large amounts of fluoride observed in beverages could be ascribed to the use of fluoridated water for their preparation as well as to tea included in the daily diet. The difference between unashed and ashed samples reflects the amount of non-ionic fluorine in the food that is converted to the ionic form during ashing. The ratio of ashed to inorganic fluoride found by Singer *et al* (1980) was in some cases lower than expected. Taves (1983) found generally good agreement between ashed and unashed fluoride while Singer and Ophaug (1986) established that the amount of total fluoride is generally higher than that of inorganic fluoride. Of all composites analyzed, the highest ratio was found in dairy products. The total fluoride content of some food items reported by Lopez and Navia (1988) agreed in most cases with those reported by Taves (1983) and the ratios of total to free fluoride were in agreement with those obtained by Singer and Ophaug (1986).

A comprehensive survey of Finnish foods revealed that fish containing 5.4 mg/kg was the commodity group containing the greatest amount of fluoride on a dry weight basis, with dairy products next at 0.90 mg/kg (Varo and Koivistoinen, 1980). Dabeka and McKenzie (1995) determined the amount of unashed (inorganic or free) fluoride in 147 food samples. Individual samples with the greatest amount of fluoride were cooked veal (1.23 mg/kg), canned fish (4.57 mg/kg), shellfish (3.36 mg/kg), cooked wheat cereal (1.02 mg/kg) and tea (4.97 mg/kg). Food groups with the highest mean fluoride amounts were fish (2.12 mg/kg), soups (6.06 mg/kg) and beverages (1.15 mg/kg). It is important to add that whether these amounts were calculated on a dry or fresh weight basis was not explicitly stated.

Wide variations in the amount of fluoride are observed in different studies and between and within food groups may be because of

a) differences in origin and growth of foodstuffs,
b) differences in manufacturing procedures,
c) type of water used for preparation and,
d) differences in the analytical procedures including sample pre-treatment procedures.

3.9.1.3 Concentration of fluoride in beverages

Increasing numbers of people are consuming beverages instead of water, so their fluoride content has to be considered when estimating total intake of fluoride. The tea plant (Camellia sinensis) is known to take up fluoride from the soil and accumulate it in the leaves, from where it is readily released during infusion (Gulati *et al.*, 1993). The fluoride content depends on the age of leaves, type of soil, soil pH and on total and extractable fluorine in the top soil (Fung *et al.*, 1999; Xie *et al.*, 2001). Total fluorine in tea leaves ranges from 100 to 880 mg/kg (Fung *et al.*, 1999). Black (fermented) and green (unfermented) tea is made from young leaves and contains a lower amount of fluoride than brick tea made from fallen and old leaves (Cao *et al.*, 2004).

A large percentage of the total fluorine 25 - 90%, is released during infusion depending on the amount of dry tea used, the granulation of the tea, the concentration of fluoride in the added water, the presence of milk and duration of infusion (Fung *et al.*, 1999; Cao *et al.*, 1998; Malde *et al.*, 2006). The fluoride content of bottled teas in Germany, United States and Taiwan were in the ranges of 0.03–1.79, 1.0–4.1 and average of 25.7 mg/L, respectively (Whyte, 2006; Lung *et al.*, 2003). Different researchers found fluoride concentration in the range of 8.8-246mg/kg in the study made on different kinds of tea infusion brewed on fluoride free and fluoride contaminated water (Lung *et al.*, 2003; Zerabruk et al., 2010; Malde et al., 2003).

Concentrations of fluoride in fruit and vegetable juices are generally low. Concentrations exceeding 1 mg/L of fluoride were determined in 18 out of 43 ready-to-drink juices in the United States, most probably due to contamination with fluoride-containing pesticides (Stannard *et al.*, 1991). Higher concentrations of fluoride in the range from 0.02 to 5.4 mg/L in juices (Kiritsy *et al.*, 1996; Njenga *et al.*, 2005) were also reported. Concentrations of fluoride in soft drinks ranged from 0.02 to 1.28 mg/L and exceeded 0.60 mg/L of fluoride for 71% of the products (Heilman *et al.*, 1999). Wines generally contain less than 1 mg/L of fluoride (Rodrı´guez *et al.*, 2003). Concentrations exceeding 1 mg/L were determined in some

Californian wines, most probably the result of using cryolite as a pesticide (Burgstahler & Robinson, 1997). The higher concentrations of fluoride found in some juices, soft drinks and beers can generally be ascribed to the naturally occurring or artificially added fluoride in water used for preparation (Warnakulasuriya *et al.*, 2002).

3.9.1.4 Milk and baby formulas

The concentration of fluoride in human milk generally ranges from 2 to 50 mg/L (Ekstrand *et al.*, 1981; Esala *et al.*, 1982; WHO/IAEA, 1989). The concentration in milk of cows fed with a normal diet was 0.103 mg/L and 0.283 mg/L in those fed on contaminated pastures (Dirks *et al.*, 1974). The concentrations of fluoride in ready-to-feed formulas in the United States and Canada range from 0.1 to 0.3 mg/L while the fluoride concentrations of powdered or liquid-concentrate infant formulas depend mainly on the concentration of fluoride in the water used to reconstitute the product (SCSEDRI, 1997).

A study on the concentration of fluoride in infant formula reconstituted with water in Australia revealed concentrations from 0.031 to 0.532 mg/L of fluoride for formulas reconstituted with water not containing fluoride, 0.131 to 0.632 mg/L of fluoride for formulas reconstituted with water containing 0.1 mg/L of fluoride and 1.031 to 1.532 mg/L if formulas were reconstituted with water containing 1.1 mg/L of fluoride (Silva and Reynolds, 1996). Concentrations of fluoride in powdered milk formulas in Brazil ranged from 0.01 to 0.75 mg/L for those prepared with deionized water, from 0.02 to 1.37 mg/L for those prepared with bottled mineral water containing 0.02–0.69 mg/L of fluoride and from 0.91 to 1.65 mg/L for formulas prepared with fluoridated drinking water containing 0.9 mg/L of fluoride (Buzalaf *et al.*, 2001).

3.9.1.5 Fluoride in Dietary supplements

Unlike controlled fluoridated drinking water and toothpastes, there is little quantitative information on the cariostatic action of fluoridated salt, although it is considered to act in a manner like that of fluoridated drinking water (WHO, 2002). Fluoridation of domestic salt for human consumption was initiated in Switzerland in 1955 (Marthaler, 2005a). Fluoridated salt usually contains 200–250 mg/kg of fluoride, mostly in the form of potassium salt (EFSA,

2005). The average daily adult salt intake is estimated to vary from 5 to 10 g (WHO, 1994) so, if all consumed salt were fluoridated, the total daily intake of fluoride would range from 1 to 2.5 mgF. Salt fluoridation can reach the entire population; however, addition of fluoride is limited mainly to domestic salt.

Schemes of fluoridation of domestic salt are most developed in France, Germany and Switzerland and adopted by other EU members (Marthaler, 2005b; Marthaler, 2005a; Tramini, 2005; Schulte, 2005; Marthaler and Pollak, 2005; Gillespie and Baez, 2005). Formerly, the administration of fluoridated milk to children was considered to be a suitable means of increasing their intake of fluoride; however, little quantitative information is available on the efficacy of this delivery system in the prevention of dental caries (WHO, 2002). Encouraging results have been reported with milk fluoridation (Twetman, 2005).

Dietary fluoride supplements, available in the form of tablets, drops, lozenges and rinse supplements are recommended for caries prevention by medical societies in some countries, especially where fluoride concentration from drinking water is low. Fluoride supplements are rarely prescribed for adults. These supplements contain different quantities of fluoride in the form of NaF (Whitford, 1987).

3.9.1.6 Fluoride from other sources: Dental products

Exposure to fluoride occurs through fluoride-containing toothpastes, gels and rinses. Fluoridated toothpastes usually contain from 1000 to 1500 mg/g of fluoride (Whitford, 1987). Because of poor control of the swallowing reflex, toothpastes for children usually contain lower amounts of fluoride from 250 to 500 mg/g (Newbrun, 1992). Yet convincing evidence for the efficacy of fluoridated toothpaste is lacking. Davies and his colleagues found (2002 that there was significantly less caries in a group receiving 1450 mg/g of fluoride toothpaste than in groups receiving 440 mg/g or fluoride-free toothpaste. Topical mouth rinses marketed for daily home use can contain between 230 and 1000 mg/L (WHO, 1994; Whitford, 1987).

3.9.2 Fluoride Intake

Accurate estimates of fluoride intake are important in order to resolve potential problems of too low or too high exposure. Drinking water, food, beverages and fluoride-containing dentifrices are regarded as the main contributors to human fluoride intake (WHO, 2002; Schamschula *et al.*, 1988; Erdal and Buchanan, 2005; Malde *et al.*, 2004).

For a given individual, water consumption increases with temperature, humidity, exercise and state of health (Murray, 1986). As a result of this total daily fluoride exposure can vary markedly from one region to another. However, from several studies, a rough estimate of total daily fluoride exposure in a temperate climate would be approximately 0.6 mg per adult per day in an area in which no fluoride is added to the drinking-water and 2 mg per adult per day in a fluoridated area (WHO, 1984). Different studies indicated that although drinking water is epidemiologically the most important source of fluoride in most areas, considerable exposure risk is also associated with food and drinks, especially tea (WHO, 1984; Malde *et al.*, 2004).

3.9.2.1 Fluoride intake of adults

The major source of fluoride intake in adults is diet (water, food and beverage). Wide variations in the total intake of fluoride within and between studies are reported (Taves, 1983; Dabeka and McKenzie, 1995; Osis *et al.*, 1974;; Dabeka *et al.*, 1987; Couzy *et al.*, 1988; Haldimann and Zimmerli, 1993; Ponikvar *et al.*, 2007; Malde *et al.*, 2011). This can be ascribed to

a) considerable differences in the amounts of fluoride in similar food items,
b) large variation in the quantities consumed,
c) differences between the age groups and genders studied and,
d) differences in the analytical techniques used

It is also important to note that estimates of quantities of foods consumed, such as standard food tables and market basket surveys, were used, rather than actual quantities of food consumed.

Several studies reported that the intake of fluoride was higher in areas with fluoridated than non-fluoridated water. The average daily total fluoride intake in non-fluoridated areas ranged from 0.56 to 1.50 mg. The daily intake of fluoride in fluoridated areas was almost twofold higher (being 0.9–3.8 mg) and even much higher in naturally fluoride contaminated areas (Dabeka *et al.*, 1987; Couzy *et al.*, 1988; Haldimann and Zimmerli, 1993; Ponikvar *et al.*, 2007; Malde *et al.*, 2011). In some areas where the fluoride content of the water is low, but the intake from food and tea is sufficiently high for the incidence of dental fluorosis to exceed 80% (Han *et al.*, 1995). Another, representative example is England, where tea consumption is traditionally high; a study found the daily average intake of fluoride from tea to be 1.26 mg in children and 2.55 mg in adults (Cook, 1969), making a significant contribution to the total daily fluoride intake.

3.9.2.2 Fluoride intake in children

Small amounts of fluoride have been proven to be effective in preventing dental caries, but excessive, chronic intake by young children can result in the development of dental fluorosis; the critical period of exposure for all permanent teeth being between 11 months and 8 years of age (Fejerskov *et al.*, 1977; Ishii and Suckling, 1991). The main sources of fluoride intake in children are diet (food, beverage and water), fluoride-containing dentifrices and fluoride-containing supplements. Intake from fluoridated dental products can be substantial, particularly in young children who have poor control of the swallowing (Baelum *et al.*, 1987).

Intake estimates were generally lower than expected in the studies where duplicate diet technique, which is the most accurate technique of estimating actual daily intake, was used. Intakes of fluoride for breastfed infants are usually negligible, because fluoride is poorly transferred from plasma to breast milk (Ekstrand *et al.*, 1981).

Fluoride ingestion from toothpastes is also common and often relatively substantial. Younger children (7 years and under) ingest, on average, 25–38% of toothpaste per brushing (Levy *et al.*, 1995). The amount of toothpaste used by children per brushing was determined to be 0.7 g on average. The amount was considerably higher than the commonly recommended 'pea size' of 0.25 g (EFSA, 2005).

3.10 Biomarkers of Fluoride Exposure and Their Status

Monitoring human exposure to fluoride can be accomplished with varying degrees of accuracy through the analysis of several biological fluids and tissues. The concentrations of fluoride in plasma, serum and urine have been considered as useful biomarkers for monitoring deficiency or excessive intake of biologically available fluoride. Concentrations of fluoride in nails, hair bones and teeth have also been used as biomarkers of exposure. However, they are ethically and technically limited for use in large-scale monitoring of the body burden of fluoride in humans.

3.10.1 Plasma, saliva and urine as contemporary markers

The levels of fluoride in body fluids (plasma, saliva, urine) give some indication of recent fluoride intake. Fluoride ion does not produce any metabolites. This indicator, however, does not well reflect the fluoride body burden or the accumulation of fluoride in the body, because the relation between fluoride concentrations in bone and in extracellular fluids is incompletely defined. The concentration of fluoride in plasma, urine, saliva and dental plaque is dependent on the intake via water, diet, fluoride supplements and fluoride-containing dentifrices (Ekstrand, 1977b; Twetman *et al.*, 1998; Stephan *et al.*, 2004).

Fluoride concentrations in ductal and glandular saliva closely follow the plasma concentration but at a lower level. A close relationship was found between the concentrations of fluoride in saliva and plasma, the ratio being 0.55: 0.80 (Oliveby, 1989; Whitford *et al.*, 1999). A commonly used indicator for fluoride exposure is its concentration in urine (Toth *et al.*, 2005). The urinary excretion rate of fluoride correlates better with its concentration in plasma (Ekstrand and Ehrnebo, 1983).

3.10.2 Nails and hair as recent markers

The concentration of fluoride in nails and hair appears to be proportional to intake over longer periods of time, taking into account their growth rate (Czarnowski *et al.*, 1996). Daily intake from food, water, dentifrices or fluoride supplements contributes fluoride body burden. The

major advantage of nails and hair over fluids and tissues as biomarkers to assess fluoride exposure is that they can easily be obtained in a non-invasive manner. In contrast to plasma, saliva and urine, whose fluoride concentrations provide a snapshot at a certain point of time and are subject to change due to recent fluoride intake and certain physiological variables, the concentration of fluoride in nails and hair is cumulative and reflects the average level of intake over a time period, but depends on how often the nails are clipped or hair cut (Kokot and Drzewiecki, 2000; Whitford, 2005).

The amount of fluoride in fingernails or toenails as reported in summary table of literature reports prepared by Whitford (2005) is about 0.5 - 5 mg/g. The average amount of fluoride found in samples of hair of the low exposure population varied from 1.3 to 2.6 mg/g, while the average values were between 400 and 2830 mg/g among aluminium workers and exhibited strong dependence on the type of work exposure to fluoride (Kokot and Drzewiecki, 2000).

3.10.3 Calcified tissues as historical markers

More than 99% of body burden fluoride is found in calcified tissues. It is generally agreed that the level of chronic exposure extending over period of years is best assessed by determining fluoride concentrations in bone, but this tissue is for obvious reasons collected only rarely. Plasma fluoride concentrations in persons who have had no fluoride intake during the previous several hours can serve as a biomarker for the chronic level of fluoride intake and the total amount of fluoride in the body (Ericsson *et al.*, 1973). Saliva could similarly reflect bone fluoride concentrations, because the ratio of the concentration of fluoride in plasma to that in saliva is relatively constant.

In contrast to skeletal bone and dentine, which accumulate fluoride throughout life and whose levels are proportional to the absorbed dose of fluoride; fluoride in enamel is not an appropriate biomarker, because most of its fluorine was taken up during tooth formation (EFSA, 2005). The post-eruptive fluoride uptake of enamel is expressed only in the outer layer and depends on the concentration of fluoride in the oral cavity (WHO, 1994).

3.11 Analytical Methods for Fluorine Determination

Monitoring the intake of fluoride and maintain it at adequate levels is important so that optimal protection against dental caries achieved, without excessive intake (Liebman & Ponikvar, 2005). Reliable analytical techniques are a prerequisite for accurate and precise determination of human exposure to fluoride. The extensive classification of different forms of fluorine in biological materials is based on the works of different researchers (Taves, 1968a) Fluorine in biological samples is present in the form of ionic or non-ionic inorganic fluoride and organic fluorine. The latter comprises covalent fluorine that is bound to carbon in all organic fluorine compounds and results from exposure to certain fluorine-containing compounds from natural and/or industrial sources (Belisle, 1981). Total fluorine includes both inorganic fluoride and organic fluorine. Methods for determining any form of fluorine in any type of material generally rely on the determination of fluorine in the form of free inorganic fluoride ion (F^-).

Four basic requirements for accurate and precise determination of the amount of fluoride or total fluorine in any type of sample include that:

(1) the sample has to be appropriately pre-treated so that the required form of fluorine can be determined;

(2) interfering reactions effectively suppressed;

(3) the final concentration of fluorine be above the detection limit of the method (sample concentrated) and

(4) method validated using certified reference material (CRM), or the results of analyses compared to the results obtained by an independent method / laboratory.

The aim of this section is to present a concise overview of separation, concentration and decomposition methods for sample pre-treatment and an overview of the analytical methods available for determining free inorganic fluoride and total fluorine in the environment al and biological materials.

3.11.1 Sample pre-treatment procedures

In the classical Willard–Winter (1933) distillation procedure, decomposition of compounds and separation of resulting fluoride from interfering substances take place concurrently. Fluoride can also be separated from interfering substances by using diffusion and microdiffusion techniques that require 6–48 h for quantitative separation of fluoride from the interfering ions (Taves, 1968b; Ikenishi, *et al.*, 1990).

Faster procedures, requiring only a few minutes, are the adsorption of fluoride on magnesium oxide or calcium phosphate (V) or a reverse extraction procedure (Venkateswarlu, 1975). Free inorganic and acid-labile fluoride are determined together by diffusion and reverse extraction, while only free inorganic fluoride is determined by the adsorption technique (Venkateswarlu, 1990). Free inorganic fluoride can be also determined directly in filtrates of aqueous solution or suspension of the sample (Ponikvar *et al.*, 2000). Organic fluorine can be determined after conversion to inorganic fluoride by a rapid procedure using sodium biphenyl, which cleaves covalent C–F bonds (Venkateswarlu, 1982).

The purpose of decomposition of the sample is to release fluorine from inorganic or organic matrixes and convert it to fluoride ions. Commonly used procedures involve oxygen bomb combustion in a closed bomb (Levaggi *et al.*, 1971) , open ashing (Esala *et al*, 1983) , alkali hydroxide or alkali carbonate fusion (Eyde, 1982; Sager, 1987; Malde *et al.*, 2001), pyrohydrolysis (Warf *et al.*, 1954; Inkielewicz *et al.*, 2003), acid extraction (Torma, 1975; Villa, 1979) and microwave acid digestion (Aysola *et al.*, 1987; Matusiewicz *et al.*, 1989; Grobler and Louw, 1998).

3.11.2 Analytical methods for determining fluoride

Numerous analytical methods have been described for determining fluorine in a variety of samples. In early methods, the sample was first decomposed, fluoride separated from interfering substances and determined using volumetric (Andersson & Gelin, 1967), spectrophotometric (Andersson & Gelin, 1967) and fluorometric (Li and Chen, 2000) methods. These methods have mostly been replaced by the introduction of the fluoride ion-selective electrode (ISE) by Frant and Ross (1966), a breakthrough method in analytical

chemistry of fluorine. Briefly, fluoride ISE is designed to determine fluoride ion activity in aqueous solutions. The observed potential is affected by the total ionic strength of the solution, the pH and the presence of any fluoride-complexing cations.

The ionic strength and pH of the solution are adjusted and interfering ions complexed by adding a background solution, the so-called TISAB (Total Ionic Strength Adjustment Buffer). As a consequence, the amount of free inorganic fluoride determined is dependent on the composition of the TISAB employed for the analysis (Liebman and Ponikvar, 2005). Fluoride ISE is a widely used technique for determining amounts of free or total fluoride because it is highly selective and covers a wide concentration range. Fluoride ion-selective microelectrodes for determining fluoride in microlitre to nanolitre volumes of samples have also been proposed (Durst and Taylor, 1967; Vogel, *et al.*, 1990).

Other frequently used methods for determining fluoride include ion chromatography (Michigami *et al.*, 1993) gas chromatography (Quintana, *et al.*, 2003) and aluminium monofluoride (AlF) molecular absorption spectrometry (Tsunoda *et al.*, 1977; Chiba *et al.*, 1980). Less frequently employed methods include enzymatic (Marcos and Townshend, 1995), catalytic (Klockow *et al.*, 1977), polarographic (Guanghan *et al.*, 1999) and voltammetric methods (Wang and Grabaric, 1990). Helium microwave-induced (Gehlhausen and Carnahan, 1989) or inductively coupled plasma atomic emission spectrometry (Manzoori and Miyazaki, 1990), electrothermal atomic absorption spectrometry (Cobo *et al.*,1993), inductively coupled plasma-mass spectrometry (Bayon *et al.*, 1999), radio-activation (Kobayashi and Shigematsu, 1987), proton-induced gamma emission (Paik *et al.*, 1997), near-infrared spectroscopy (Grummisch *et al.*, 1998) and neutron activation analysis (Cheng *et al.*, 1997) are also some of the the possible methods for fluoride analysis.

3.11.3 Determining fluoride in specific types of materials

3.11.3.1 Fluoride in environmental media

The concentration of fluoride in water can usually be determined directly without pre-treatment. Among the numerous published analytical techniques, potentiometry with fluoride ISE, ion chromatography, and spectrophotometry are commonly used. If the amount of

fluoride present in water is very low, pre-concentration may be required. Determination of total fluoride in soil, sediments, oxides and other raw materials requires complete decomposition of the sample to release bound fluoride. Accumulation of fluoride in soil might be studied by employing appropriate extraction procedures (Venkateswarlu, 1994).

3.11.3.2 Fluoride in biological tissues, fluids and related materials

Approaches to determining different forms of fluorine in biological and related materials have been reviewed extensively by Venkateswarlu (1990). Total decomposition is a general pre-requisite for determining the amount of total fluorine in biological materials other than urine (Venkateswarlu, 1994; Dean, 1942). The difference between total fluorine and inorganic fluoride in biological and related materials usually represents organic fluorine. Among the various published decomposition procedures, the most important are open ashing, fusion, oxygen combustion and digestion, followed by a separation or concentration step.

3.11.3.3 Fluoride in supplements and dental products

Total fluorine in fluoride supplements and dental products could be determined with minimal samples pre-treatment as for example by direct acid extraction or heating in TISAB buffer solution and subsequent determination of fluoride using fluoride ISE for the reason that entire fluorine, in supplements and dental products are free inorganic fluoride.

3.12 Fluoride Intake and Its Risk Assessment

Since1940's it has been recognized and promoted that fluoride has both beneficial and harmful effects on dental health. Small amounts of fluoride have been proven to be beneficial in preventing dental caries because of the greater resistance of enamel-containing fluoride to ingested acids or to acids generated by oral bacteria. Moreover fluoride also inhibits sugar metabolism in oral bacteria (Dean, 1942; EFSA, 2005). On the other hand, excessive intake of fluoride during enamel maturation, from birth to 7–8 years of age can lead to the development of dental fluorosis of deciduous, but predominantly of permanent teeth. Excessive fluoride intake by children older than 7–8 years does not cause dental fluorosis (Fawell *et al.*, 2006).

Fluoride is also believed to have a unique ability to stimulate new bone formation. The actual mechanism of fluoride action is still a subject of debate; however, it is more recently believed that the majority of the benefit from fluoride can be attributed to its topical, rather than systemic, effects (Hellwig and Lennon, 2004). A conflict arises from two claims: the first, that fluoride stimulates new bone growth and hence is useful therapeutically in controlling osteoporosis and the second is that it is the cause of the increasing prevalence of hip fractures in the elderly (OSC, 2003; WHO. 1994). Fluoride is currently not recommended for the treatment of osteoporosis, although slow release fluoride therapy is reportedly beneficial (OSC, 2003). The long-term benefit of the latter is unknown (Beers and. Berkow, 1999). Fluorine is available to humans, plants and animals mainly in the form of fluoride ion (F^-). Body fluoride status depends on numerous factors, including the total amount of fluoride ingested daily, its bioavailability and metabolism. The adequate intake (AI) of fluoride from all sources is set by the Standing Committee on the Scientific Evaluation of the Dietary Reference Intakes (SCSEDRI) at 0.05 mg/day/kg body weight; this intake is recommended for all ages above 6 months, because it confers a high level of protection against dental caries and is not associated with any known unwanted health effects (SCSEDRI, 1997).

Drinking water, beverages and fluoride-containing dentifrices are regarded as the main dietary contributors to human fluoride intake. Food has more recently being recognized as a potentially important source of fluoride (Malde *et al.,* 1997; Susheela, 2002). A major source of fluoride in some areas arises from its release into the environment by coal combustion, in process waters and waste from various industrial processes. Because of the low natural levels of fluoride in some water supplies and correspondingly high levels of dental caries, many authorities worldwide have permitted fluoridation of water supplies, although this has met some opposition, partly because of the potential health or dental effects including fluorosis.

Much research has been devoted to fluoride and its health effects, but, although the benefits and risks of exposure to fluoride are well known, it is still not possible to make a firm distinction between a safe daily dose and a potentially harmful one. Thresholds of 0.05–0.07 and 0.03–0.04 mg/day/kg body weight of fluoride have both been suggested for the

appearance of dental fluorosis (Fejerskov et *al.*, 1987; Baelum et *al.*, 1987; Burt, 1992). It is not surprising that few subjects in medicine have proved more controversial than fluoridation of public water supplies, although supported by the World Health Organization (WHO) among other organizations (WHO, 1994). The controversy is illustrated by the fact that, while the U.S. Centers for Disease Control and Prevention (CDC) claimed that water fluoridation is 1 of the 10 great public health achievements in the United States during the 20th century (CDC, 2001) and nearly two-thirds of the population receives fluoridated drinking water (CDC, 2002) while water fluoridation is banned in most of Europe (Cross and Carton, 2003). The current state of knowledge among many researchers about fluoride is that the effect of a substance depends on the dosage regimen. As Paracelsus (1493–1541) said, 'All substances are poisons; there is none which is not a poison. The right dose differentiates a poison and a remedy'. Therefore determining the right dose which will not compromise the health of the community is very important.

3.12.1 Dietary intake assessment

Dietary assessments are performed for three main objectives, namely: to assess mean intakes of a group, to determine the prevalence of inadequate intake, and to establish a relationship between dietary intake and laboratory or functional outcomes (Winichagoon, 2008). The dietary intake of fluoride is determined based on the later objective; to investigate if the intake level is above recommended daily allowance which otherwise cause fluorosis. Of the dietary assessment methods, duplicate plate method is selected by different researchers to determine the daily fluoride intake from dietary sources (Malde et al., 2003).

3.12.2 Fluoride Health Risk Assessment

According to Connell (2005), hazard identification, exposure assessment, determination of toxicity and risk characterization are the most important steps in the human health risk assessment procedure (on dose response relations). To characterize the risk quantitatively, one need to know the exposure to the chemical and the potency of the adverse effect of the chemical. In this regard, the most common toxicity data used in evaluating health effects are the no observable adverse effect level (NOAEL) and least observable adverse effect level (LOAEL) values of the chemical. In general, the health risk of a chemical is entirely related to

the dose or exposure of the chemical, its bioavailability and the potency of its adverse effect (Connell, 2005).

In the health risk assessment, there are two principal sources of information on health effects resulting from exposure to chemicals that can be used in deriving guideline values. The first is studies on human populations. The value of such investigations is often limited, owing to lack of quantitative information on the concentrations to which people are exposed or on simultaneous exposure to other agents. The second, and the one used most often, is toxicity studies using laboratory animals. Such studies are generally limited because of the relatively small numbers of animals used and the relatively high doses administered. Furthermore, there is a need to extrapolate the results to the low doses to which human populations are usually exposed (EFSA, 2008b).

In order to derive a guideline value to protect human health, it is necessary to select the most suitable experimental animal study on which to base the extrapolation. Data from well-conducted studies, where a clear dose-response relationship has been demonstrated, are preferred. Expert judgment was exercised in the selection of the most appropriate study from the range of information available.

3.12.3 Drinking-water consumption and body weight

Global data on the consumption of drinking-water are limited. In studies carried out in Canada, the Netherlands, the United Kingdom, and the United States of America, the average daily *per capita* consumption was usually found to be less than 2 litres, but there was considerable variation between individuals. As water intake is likely to vary with climate, physical activity, and culture, the above studies, which were conducted in temperate zones, can give only a limited view of consumption patterns throughout the world. At temperatures above 25°C, for example, there is a sharp rise in fluid intake, largely to meet the demands of an increased sweat rate. In developing the guideline values for potentially hazardous chemicals, a daily *per capita* consumption of 2 litres by a person weighing 60 kg was generally assumed. Such an assumption may underestimate the consumption of water per unit

weight, and thus exposure, for those living in hot climates as well as for infants and children, who consume more fluid per unit weight than adults.

The higher intakes, and hence exposure, for infants and children apply for only a limited time, but this period may coincide with greater sensitivity to some toxic agents and less for others. Where it was judged that this segment of the population was at a particularly high risk from exposure to certain chemicals, the guideline value was derived on the basis of a 10-kg child consuming 1 litre per day or a 5-kg infant consuming 0.75 litre per day. The corresponding daily fluid intakes are higher than for adults on a body weight basis.

For most kinds of toxicity, it is generally believed that there is a dose below which no adverse effects will occur. For chemicals that give rise to such toxic effects, a tolerable daily intake (TDI) can be derived as follows:

TDI (Tolerable Daily Intake) is the amount of a chemical that can be taken daily by humans in a safe manner. It is calculated by dividing the NOAEL (No Observed Adverse Effect Level, determined in toxicity tests, etc.) by product of Safety Factor (SF) / Uncertainty Factors (UFs) and converted to the NOAEL for humans.

$$\text{TDI} = \frac{NOAEL}{UF}$$

where:

- $NOAEL$ = no-observed-adverse-effect level,
- $LOAEL$ = lowest-observed-adverse-effect level,
- UF = Uncertainty factor or Safety factor

Hazard Quotient: Risk assessment generally uses factors such as Hazard Quotient (HQ) and Margin of Exposure (MOE). Both factors are based on the same concepts. HQ compares the magnitude of Estimated Human Exposure (EHE) with that of TDI (Tolerable Daily Intake). If HQ is larger than 1, i.e., EHE exceeds TDI, there is risk. If HQ is smaller than 1, i.e., if EHE does not exceed TDI, there is no risk.

$$\text{HQ (Hazard Quotient)} = \frac{\text{EHE (Estimated Human Exposure) or Dose}}{\text{TDI (Tolerable Daily Intake)}}$$

If HQ (Hazard Quotient) ≥ 1: Risk

If HQ (Hazard Quotient) < 1: No Risk

Daily Intake (DI): the daily intake considers all the main dietary sources of the contaminant. Daily intake is calculated per body weight of the organism per day as follows:

$$\text{DI} = \frac{CABA\,(w)}{BW} + \frac{(CABA\,(f)}{BW}$$

Where: DI is daily intake in mg/kg bw/day, C fluoride concentration in mg/kg in food or water, A total amount of water or food consumed in kg, BA bioavailability of fluoride in drinking water or food, BW individuals body weight in kg (Connell, 2005).

3.12.4 Derivation of guideline values using a tolerable daily intake approach

The guideline value (GV) is derived from the TDI as follows:

$$GV = \frac{TDI\ x\ bw\ x\ P}{C}$$

Where:

- bw = body weight (60 kg for adults, 10 kg for children),
- P = fraction of the TDI allocated to drinking-water,
- C = daily drinking-water consumption (2 liters for adults, 1 liter for children).

3.12.5 Risk Assessment using Margin of Exposure (MOE)

MOE compares the magnitude of NOAEL (No Observed Adverse Effect Level) with that of EHE (Estimated Human Exposure). However the NOAEL is determined by toxicity tests so the calculation of MOE does not incorporate conversion into humans (it does not take

uncertainty into consideration). MOE needs to be compared with product of Uncertainty Factors (UFs). If MOE is smaller than UFs, there is risk of the toxicant and if MOE is larger than UFs, there is no fear of risk.

Margin of Exposure (MOE) is calculated according to the following equation:

$$MOE = \frac{\text{NOAEL (No Observed Adverse Effect Level)}}{\text{EHE (Estimated Human Exposure)}}$$

If MOE (Margin Of Exposure) ≤ UFs (product of Uncertainty Factors)	Risk
If MOE (Margin Of Exposure) > UFs (product of Uncertainty Factors)	No Risk

We may say that larger the UFs (product of Uncertainty Factors) are, the less reliable the assessment results become.

3.12.6 Differences in Risk Assessment methods

HQ (Hazard Quotient) includes UFs (product of Uncertainty Factors). Risk Assessment is evaluated against 1 as evaluation standard when HQ is used in the calculation. HQ (Hazard Quotient) is advantageous in that whether or not there is risk can be simply determined only by checking whether HQ is larger or smaller than 1. MOE on the other hand has an advantageous of employing numerical analysis of uncertainty in Risk Assessment. In addition it shows clear differences by improving reliability, i.e., makes clear whether a judgment of "possible hazards" is attributable to a shortage of information (resulting in high UFs) or to the availability of information that is to some extent reliable.

3.13 Guideline values of fluoride in water

WHO (Fawell *et al.*, 2006) has set an upper drinking water quality guideline of 1.5 mgF-/L.

Conversely, WHO also recommends intakes of water containing 0.5–1.0 mg/L in the prevention of dental caries. Ingestion of water with fluoride concentrations above 1.5 mg/L results in dental fluorosis characterized initially by opaque white patches, staining, mottling and pitting of teeth. WHO also noted that drinking water with 1.5, 3.0-6.0 and >10 mg/L fluoride level causes; mottling of teeth (very occasionally), skeletal fluorosis and crippling fluorosis on humans (WHO, 1984). However, WHO emphasizes that in setting national standards for fluoride it is particularly important to consider climatic conditions, volumes of water intake, and intake of fluoride from other sources (e.g. food and others) (WHO, 2006). Accordingly, different countries have developed different guideline values for fluoride. For example, upper tolerable intake levels for fluoride have been established in Europe amounting to 1.5 mg/day for 1–3 year old children, 2.5 mg/day for 4–8 year old children, 5 mg/day for 9–15 year old children and 7 mg/day for adults (≥ 15 year old). These upper tolerable intake levels apply to fluoride intake from water, beverages and foodstuffs including fluoridated salt, dental health products and fluoride tablets for caries prevention (EFSA, 2005).

On the other hand, the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) in the UK considered an intake of 0.05 mg/kg bw/day to be a no observable adverse effect level (NOAEL) for moderate dental fluorosis (COT, 2003), even though the Committee pointed out that the threshold dose at which fluoride causes moderate dental fluorosis was 0.1 mg/kg bw/day. According to CDA (2008), the total daily fluoride intake from all sources should not exceed 0.05-0.07 mg F/kg body weight in order to minimize the risk of dental fluorosis. The prevalence of moderate dental fluorosis of permanent teeth is less than 5% in populations ingesting 0.08-0.12 mg/kg bw/day (US EPA, 2010).

The combination of the drinking water and dietary estimates are the basis for the inorganic fluoride Reference Dose (RfD) estimate of 0.08 mg F/kg/day. The RfD is an estimate of the fluoride dose that will protect against severe dental fluorosis, clinical stage II skeletal fluorosis and skeletal fractures while allowing for a fluoride exposure adequate to protect against tooth decay for children and adults (US EPA, 2010).

US EPA (2010) also identified a point of departure (POD) of 1.87 mg F/L for severe dental fluorosis based on benchmark dose modeling of the prevalence for severe dental fluorosis associated with specific drinking water fluoride concentrations. The POD is a lower confidence bound on the concentration in drinking water associated with severe dental fluorosis in 0.5% of the population studied. This value is consistent with other analyses of the Dean (1942) data set that identify 2 mg/L as the threshold drinking water fluoride concentration for severe dental fluorosis (US EPA, 1986; NRC, 2006).

The total daily intake of fluoride for optimal dental benefits should be 0.05 to 0.07 mg F/kg bw/day and no more than 0.1 mg/kg bw/day to avoid the risk of dental fluorosis (AAP-CN) 1986). The dietary fluoride intake excess of 0.1 mg/kg body weight has been generally accepted to cause dental fluorosis and intake levels from 0.05 to 0.07 mg/kg body weight is optimal for dental health of children from 1-12 years (Devika and Nagendra, 2009).

Table 1: Level of fluoride in drinking water and food versus its human health effect.

Fluoride Concen	Media	Effects
1 ppm	Water	Dental Caries reduction
2 ppm or <2 ppm	Water	Mottled Enamel (dental fluorosis)
8 ppm	Water	10 % osteosclerosis
20 – 80 mg/day	Water or food	Crippling Skeletal fluorosis
50 ppm	Water or food	Thyroid changes
100 ppm	Water or food	Growth retardation
125 ppm	Water or food	Kidney changes
2.5 – 5 g		Acute dose Death

Source: WHO, 1970.

To produce signs of acute fluoride intoxication, it is estimated that minimum oral doses of at least 1 mg fluoride per kg of body weight are required (WHO, 1996). Indeed, such doses could be expected from water with a fluoride content of approximately 30 mg/L. The Scientific Committee on Cosmetic Product and Non-Food Products (SCCNFP) concluded that the threshold that could cause serious symptoms and need immediate emergency treatment is 5 mg/kg bw/day for children less than 6 years of age (SCCNFP, 2003).

In Ethiopia, although it is not supported with adequate scientific explanations, Ministry of Water and Energy (MoWE) had recommended the maximum tolerance limit of fluoride in drinking water to be 3mg/L, Similarly the Ethiopian Environmental Protection Agency (EEPA) also set maximum tolerance limit of 2 mg/L (MoWR 2001). The arguments used by the MoWE for their relatively higher fluoride guideline value is that mild fluorosis (e.g. dental) is preferable to the risk posed by use of microbial contaminated surface water or the hardship imposed by traveling many kilometers to an alternative water sources. On the other hand, Ethiopian Quality and Standards Authority have virtually recommended the maximum permissible level of fluoride in drinking water to be 1.5 mg/L (ES 261, 2001).

3.14 Prevention and Mitigation of Fluorosis

3.14.1 Defluoridation

The primary preferred option is to find a supply of safe drinking-water with safe fluoride levels. Where access to safe water is already limited, de-fluoridation may be sought as a solution. But removal of excessive fluoride from drinking-water is difficult and expensive. Since the 1930s most research efforts have been directed at defluoridation of high fluoride containing water as fluorosis prevention strategies. Although several fluorosis prevention methods are technically feasible and can routinely be carried out in central water distribution systems, only few of the defluoridation methods are successfully applied on a routine basis (Dahi 1999). Most defluoridation methods are complicated and/or expensive, unproven and unreliable under field conditions in developing countries because of use of materials of questionable supply and inappropriate technology, ineffective at community level, their social acceptability and lack of sustained government and community commitments. Since all methods produce sludge with very high concentration of fluoride that has to be disposed of, only water for drinking and cooking purposes should be treated.

The more promising methods which have been field-tested include the granulated bone media and heat-activated bone char, clay and clay pot chip (Bjorvatn *et al.*, 1995; Dahi 1999).

activated alumina adsorption (Shifera & Tekle Haimanot 1999) and Nalgonda methods (WHO, 1994).

3.14.2 Malnutrition and fluorosis

Malnutrition is the cause of mental and a number of physical ailments (Gibney *et al.*, 2009). Bone deformities due to fluorosis are also reported to be more prevalent among children having higher grades of malnutrition. Different research reported from India show the critical role of malnutrition and poverty on the incidence and severity of fluorosis (Teotia *et al.*, 1984, Susheela *et al.*, 2002). Comparison of dietary adequacy, water fluoride levels, and incidence of skeletal fluorosis suggested that vitamin C deficiency (Khandare *et al.*, 2000) and poor nutrition play a major role in fluorosis (Mishra *et al.*, 1992). The study by Jolly *et al.* (1974) addressed the role of nutritional factors relative to the different clinical patterns of fluorosis. Low-calcium intake could also be another factor responsible for aggravating the problem of genu valgum (knock knee – a form of skeletal fluorosis) (Zang *et al.*, 1996). Apart from calcium, other nutrients also affect resistance or susceptibility to skeletal fluorosis as reported by Chakma *et al.* (2000).

3.14.2.1 Nutritional Counseling

The management of Fluorosis in the community can be effective if nutritional supplementation focusing on adequate intake of calcium, vitamins (C and E) and other antioxidants, is advocated and practiced simultaneously with consuming safe drinking water (Susheela and Bhatnagar, 2002). Nutrient supplementation through a dietary regime has been found to be the best approach and it is sustainable. During counseling for nutritional supplementation, it is necessary to emphasize avoiding of food items with high fluoride content and those foods which could be used for mitigation of ingested fluoride. Foods that should be avoided in endemic areas include: black rock salt / magadi, black tea (tea without milk), chewing tobacco, fluoride-containing toothpastes, mouth rinses, varnishes and other items that are commercially marketed.

Diet counseling is extremely important and the messages conveyed should be practiced by the community whether they are educated/ uneducated. Minor details also need to be conveyed, so that the community understands what items should be consumed that will provide them with essential nutrients. The focus in nutritional counseling should be to ensure that the community is aware of food substances or products locally available that should be consumed to ensure that the daily diet has all essential nutrients used to minimize the risk of fluorosis. It is also necessary to inform the community about how these nutrients can be incorporated in to different recipes to give variety to daily diet and ensure adequate intake of essential nutrients. Affordability and sustainability should also be kept in mind when counseling.

3.15 Fluorosis in Ethiopia

3.15.1 Fluoride distribution in Ethiopia

Fluoride has long been a recognized in Ethiopia, as in other parts of the East African Rift (Tekele-Haimanot *et al.*, 1987). The region of Ethiopian Rift Valley has an elevation which varies between 500 and 1,800 meters above sea level. The rift area is characterized by hot and dry climate with an average temperature of 23°C (ranges from 15°C to 38°C). In most parts of this region, ground water contains very high concentrations of naturally occurring fluoride beyond WHO guideline value for drinking water. Fluorosis is an endemic health problem in the Ethiopian Rift valley in general (Tekele-Haimanot *et al.*, 1987). Although the highest concentration and distribution of fluoride is being in the Rift System, concentrations of fluoride greater than the WHO guideline value of 1.5 mg/l have been found in ground waters from several parts of Ethiopia (Kloos and Haimanot, 1999). Some water sources, ground waters from volcanic rocks in the highlands have also reported to have concentrations above the WHO recommendation (Chernet and Eshete, 1982).

In the Ethiopian Rift, as elsewhere along the East African Rift system, hot springs and alkaline (and saline) lakes are noted to have particularly high fluoride concentrations. According to study from Gizaw (1996) on waters in the Lakes District of the southern Rift, fluoride concentrations exceed up to 60 mg/L in hot springs and ground waters from deep geothermal wells (temperatures in excess of 40°C), and even reach up to 200 mg/L in some of

Ethiopia's alkaline lakes like Chitu, Shalla and Abiyata. The high concentrations of fluoride and dissolved salts in the lakes are mainly caused by evaporation. In the same study the author on the contrary noted that hot springs from the Afar Depression in the north were of sodium-chloride composition and had low fluoride concentrations. Kloos and Haimanot (1999) on the other hand explained the availability of low fluoride concentrations in ground waters from wells and springs in the Rift Valley (example Arbaminch town) due to inputs of low-fluoride runoff from the highlands or from nearby rivers and lakes (Figure 5).

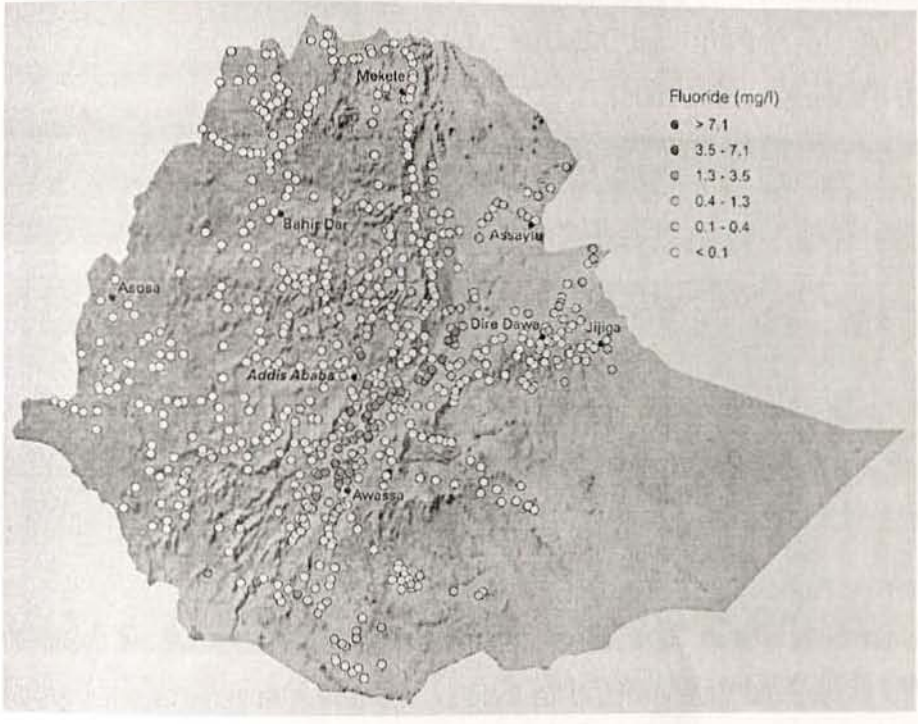

Figure 5: Fluoride distribution in water in Ethiopia (RiPPLE, 2008))

The study carried out by Ashley and Burley (1994) to investigate fluoride free water source revealed that some relationship exist between groundwater temperature and fluoride concentration. The study further added that the problematic fluoride concentrations were derived from hot springs and weathering of the volcanic bedrocks.

3.15.2 Dental and Skeletal fluorosis in Ethiopia

Ethiopian Rift Valley Region covers all or parts of Afar, Oromia and the Southern Nations, Nationalities and Peoples Regions (SNNPR). As a result of the long-term use of high-fluoride drinking water, both dental and skeletal fluorosis is common in Ethiopian Rift Valley. The severe cases of skeletal fluorosis (32%) were reported from study at Wonji (Tekle-Haimanot *et al.*, 1987). Same study reported that the maximum prevalence is seen in the 10–14 years old age-group. Kloos and Haimanot (1999) found dental fluorosis in some highland communities where the water is abstracted from volcanic rocks. Regionally 42% of groundwater sources tested in Oromia, 30% in SNNPR and 12% in Afar Region have excessive fluoride concentrations (Tekle-Haimanot *et al.*, 2006).

Figure 6: Dental fluorosis pictures in the study areas

In this region, dental mottling (Figure 6) has been recognized even in areas with fluoride concentrations in water as low as 2 mg/L (Tekele-Haimanot *et al.*, 1987; Wondwossen *et al.*, 2004a). As noted by NRC (2006), the weight of evidence indicates that the threshold for severe dental fluorosis occurs at a water fluoride level of about 2 mg/L. In warmer areas, because of the greater amounts of water consumed, dental fluorosis can also occur at lower concentrations in the drinking-water (Cao, 1992). Ever increasing number of the population, financial constraints, pollution of surface water and lack of appropriate technology for defluoridation is posing pressure on water quality management in Ethiopia (Reimann *et al.*, 2002 and 2003).

(Tekele-Haimanot, 2005)

Figure 7: Skeletal fluorosis in ERV

3.15.3 Efforts in mitigation of fluorosis in Ethiopia

Many endeavors have been recently undertaken by the National Fluorosis Mitigation Project Office, which was established by the Ministry of water and energy (MoWE) at federal and regional level to study the mitigation of fluorosis, hydrochemistry and genesis of high fluoride groundwater as well as alternative technologies of defluoridation (Figure 8). Especially the natural fluoride pollution of ground water, which remains the main source of water supply in the region now draws MoWE's attention to the Rift Valley. Therefore, currently there is strong commitment to minimize the peril of fluorosis over the quality of drinking water and the need to revise and establish new fluoride guideline value in drinking water sources that can insure its safety. An effort to address the problem of fluoride in Ethiopian drinking water supply is being led by National Fluorosis Mitigation Project Office (NFMPO) (MoWE, 2010).

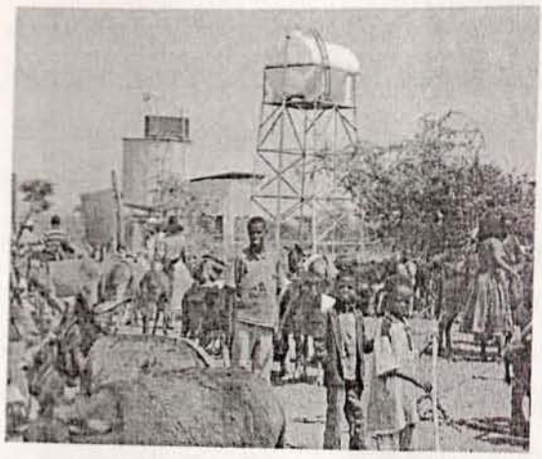

Figure 8: Defluoridation efforts in Bora woreda

On the other hand, although drinking water is likely to be the dominant pathway of fluoride exposure in Ethiopian Rift Valley areas, foodstuffs prepared with high fluoride cooking water is also an additional pathway (Malde *et al.*, 2004). The fluoride content of a dish depends on the fluoride content of the food ingredients, the fluoride concentration of the water, and the amount of water used and retained in the food during preparation (Malde *et al.*, 2004). Recent studies by Cao *et al.* (2006) also identified food as a potential hazard and states that food consumption may increase the risk of fluorosis. Khan *et al* (2004) on the other hand, proposed that each country should calculate its own optimal level of fluoride in drinking water based on the dose response relationship of fluoride in drinking water and the levels of caries and fluorosis. It is plausible to estimate the amount of fluoride ingested from all environmental and dietary sources so that rational and scientifically sound decisions can be made when guidelines for the use of fluorides are established or periodically reviewed and modified (Pang, 1992).

Unfortunately, there is no study made on total daily fluoride intake value in Ethiopia, except the study made by Malde *et al.* (2004), on dietary fluoride intake in children below 5 years of age, living in a high-fluoride area of Wonji Shoa Sugar Estate. As a result of this, there is no adequate scientific based information on the magnitude of fluoride exposure through dietary sources in the Ethiopian Rift Valley Region.

The problem is further aggravated by limited budgets which restricted the feasibility of defluoridation technologies, running cost of those established ones and inability of provision of alternative water sources.

Figure 9: Water scarcity in ERV

In addition, since the economic cost of endemic fluorosis to human beings is largely indirect and the disease is not acute, it is unlikely that fluorosis would be recognized as an area of immediate need by the government and stakeholders in developing countries. In the presence of infectious and acute diseases it is impossible to consider fluorosis as priority public health problem in the areas. In some places in Ethiopian rift valley, whatever the chemical content is, the presence of water is considered as a blessing (Figure 9).

CHAPTER FOUR: MATERIALS AND METHODS

4.1 Study Design

The study was designed in three phases: reconnaissance survey, dietary intake vs assessment of fluorosis and mitigation of ingested fluoride using nutrient supplementation (animal and human). The activities conducted in each phase are outlined in table 2.

Table 2: summary of study design, samples collected, subjects and study sites

Phase one	Phase two	Phase three
Reconnaissance survey **Samples collected** - Water - Food (prepared) - Food ingredients **Data collected** - Information about community water source (woreda) - Dietary habits **Study site selection:** Forty eight water sources were purposively selected from three dietary areas (dairy, cereal one and cereal two) in Ethiopian rift valley based on information obtained from regional water resource on fluoride level	**Dietary fluoride and Fluorosis assessment** **Samples collected** *(from the selected sites)* - Duplicate plates for children and mothers (Food, Beverages, Water and Milk) - Morning urine **Study site:** Three water sources each from dairy, cereal one and cereal two based with fluoride level in the range that causes skeletal fluorosis were selected (Benti, Kobochobare and Halaku)	**Nutrient supplementation and mitigation of ingested fluoride** **Samples collected** **Animal study (6 weeks)** - Food samples (CSB) - Urine (24hr) - Feces (24 hr) - Water **Human Trial (7 days)** - Morning urine **Study site:** Animal study in EPHI laboratory and Human study in Halaku community

4.1.1 Phase One: Reconnaissance Survey

4.1.1.1 Study area

The reconnaissance survey was conducted in main Ethiopian Rift Valley (MERV) in 2011. The Ethiopian Rift Valley is part of the biggest rift system of the world, which extends from the eastern part of Jordan to the Coast of Mozambique. The Ethiopian Rift Valley (ERV) has an average width of about 100 km and disects the country from the northeast to the southwest. Most of Ethiopian lakes are situated in the rift valley (Reimann *et al.* 2003) and contained fluoride above the WHO recommendation.

4.1.1.2 Site selection for dietary fluoride intake assessment

A preliminary study was conducted on 48 water sources purposively selected to identify three representative communities each from dairy based, cereal based and roots and tuber based areas. The aim of the survey was to identify three communities which had a water source with fluoride content in the range that causes skeletal fluorosis (5-7mg/L) but differed in dietary habits. Prior to conducting a visit to the study area, collections of relevant information regarding water fluoride level and fluorosis were carried out from regional bureau of water resources in SNNPR and Oromia. Water sources with at least 10 years' service and fluoride level ≥ 5 mg/L were selected. As much as possible, factors such as socioeconomic status and religion have been made similar in the course of the selection. During the survey food and food ingredients were collected from each area to investigate the fluoride content (Table 3). Based on the assessment, Benti water source (F = 6.2 mg/l) (8`54`N & 40`00``E) from Fentale (Fentale woreda, Oromia), Halaku water source (F = 5.0 mg/l) (7`52``N & 38`40``E) from Adamitulu (Adamitulu Jido-Kombolcha woreda, Oromia) and Kobochobare water source (F = 6.9 mg/l) (7` 17``N & 38` 06``E) from Alaba (Alaba Special woreda, SNNPR) were selected for the dietary assessment (Figure 10). During the course of selection due to change of the water source to low fluoride borehole, community with fluoride water source in the range of 5-7mg/L was not found in root and tuber based dietary areas. Hence, the root and tuber area was substituted to finger millet and corn based dietary areas.

4.1.1.3 Food and water Sampling

Water samples were collected in cleaned polyethylene bottle directly from sources. Staple foods; Injera, bread (both commercial yeast and cultural yeast raised) and unleavened bread made by the water sources were collected all along the main Ethiopian Rift Valley (ERV) from Dubti in Afar to Dimtu in Wolayita. Samples of food ingredients (grains, vegetables, legumes and tea leaves) were collected from households and community shopping centers into polyethylene bag and kept in ice box until brought to laboratory. To examine the fluoride level of tea brewed with fluoride contaminated water, 2.5 g of dry tea leaf was boiled for 3 minutes in 100ml water fluoridated at 5mgF^-/L. The final water volume made up to 100 mL in order to compensate for amount evaporated. The summary of food and food ingredients

collected are given in Table 3.

Table 3: Summary of samples collected from selected sites of Ethiopian Rift Valley (ERV)

Region	Water	Grains	Vegetable	Injera	Bread	Unleavened bread	Legumes	Tea leaves	Milk	Oil seeds
Dairy based area, Afar and Kereyu, Northern ERV** (N =11)	11	1	0	5	5	2	0	0	1	0
Maize and Wheat based area, Central ERV, Oromia (N = 22)	22	13	27	20	19	3	1	8	1	0
Finger millet and maize based area, Southern ERV, SNNPR* (N = 15)	15	7	13	9	4	10	5	2	1	2
Total	48	21	40	34	28	15	6	10	3	2

SNNPR* = South Nation Nationalities People Region, ERV** = Ethiopian Rift Valley

4.1.2 Phase Two: Dietary fluoride intake and fluorosis assessment

4.1.2.1 Daily fluoride intake by school age children (10-15years) and biological mothers

Study sites

The study areas were selected based on dietary habits and fluoride content in community water source. Some foods are believed to reduce fluoride bioavailability. Few studies have correlated mitigation of ingested fluoride by calcium rich foods such as dairy products. In this study three dietary areas, each from dairy based, maize and wheat based and finger millet and maize based were selected. The selected dietary areas have drinking water fluoride content in the range of 5-7mg/L and have similar ecological condition.

Fentale (Benti water source (8`54`N & 40`00``E): is in the North Eastern Oromia, characterized by high annual mean temperature, low rain fall and breeding of cattle (Agro pastoralist). The diet is fully dependent on dairy products.

Adamitulu (Halaku water source (7`52``N & 38`40``E): is situated in central Oromia, East Showa. It is characterized by wheat and maize growing, low annual rain fall and high annual mean temperature.

Alaba (Kobo Chobare water source (7` 17``N & 38` 06``E): is found in Alaba Special Woreda in SNNPR. The area is characterized by finger millet and maize consumption, high rain fall and relatively low annual mean temperature compared to the other two selected areas.

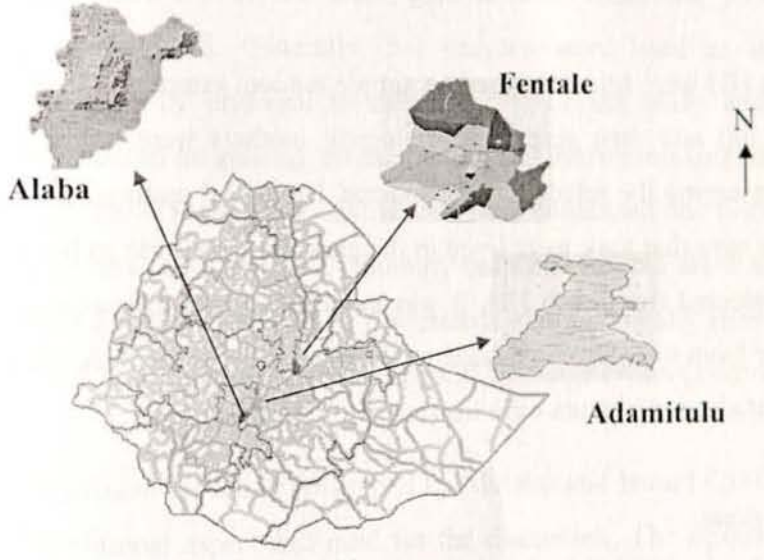

Figure10: Study sites for fluoride and fluorosis assessment

Selection of Households and Study Subjects for daily fluoride intake and fluorosis assessment

Households (HH) who had been using the selected water source, dwelled in the area for more than 10 years and had a child in the age range of 10-15 years were selected for the study. Eligible HHs were briefed about the study and the study participants including head of the HH were requested for willingness to participate in the study. All households who gave consent and eligible were recruited.

Selections of 10 to 15 years old children as study participants were based on previous reports. Tekele-Haimanot *et al.* (1987) indicated that maximum prevalence of dental fluorosis was observed in the 10–14 years old children and severe dental mottling was found in 32 % of the sampled children. The cumulative effects of fluoride exposure would be expected to be seen most clearly in children 12-14 years old at a time when most of the permanent dentition is fully erupted. Dental assessment in younger age groups may not provide sufficient data for a dose-response analysis (Dean, 1942). According to Wondwosen and his colleagues, the prevalence of dental fluorosis (TF- score $\geq$ 1) in 12 to 15 years old Ethiopian children consuming drinking water of 0.3-2.2 mg/L fluoride level was found to be 91.8% (Wondwossen *et al.*, 2004b). But for the assessment of skeletal fluorosis adults who lived for more than 10 years are selected as it is cumulative problem.

Children in each HH were selected based on simple random sampling. A total of 296 children 10 to 15 years old and their respective biological mothers were randomly selected from communities consuming the selected water sources. Biological mothers in the age range were selected to make sure that they were lived in the area and gave birth to the index child. Only one child was selected from each HH. It was strictly assessed that selected child had been consuming water from the aforementioned water source right from birth. Before assessing for dental fluorosis study participants were instructed to clean their teeth.

24 hr Duplicate plate

Food consumed by the child (10-15 years) and biological mother was collected from 20 % of HHs interviewed. During food collection, duplicate plate method was used i.e., equal amount of food consumed in each meal by a study participant over 24 hour was sampled in each HH. The HHs for duplicate plates were randomly selected. The estimated amount of food left over from amount served was reduced from the duplicate plate by care-giver. The estimated amount consumed was weighed and collected into plastic bag. The foods were collected on one week-day and one weekend. Unintentionally, the selected dietary areas are Muslim inhabitants and have no special day in the week which could have change their dietary pattern. The food ingredients were labeled with unique code and kept in cooled ice box until brought to laboratory. The pooled/composite 24 hr foods were homogenized and analyzed in

duplicates for dietary fluoride and nutrient intake. Beverages, milk and water consumed by the study participants were sampled and analyzed for dietary fluoride contribution. In order to calculate nutritional status and fluoride intake per day per body weight, the weight of the participants were recorded.

4.1.2.2 KAP of the community on fluorosis and mitigation: Protocol for qualitative data collection

The qualitative research method utilized focus group discussions (FGDs) as a technique of data collection. Participants of the FGDs were selected from the community based on their years of lived in the area, age, social activity and sex. In each group, community administration, elders (both women and men), gate keepers (influential persons) and health extension workers participated. Generally, 5-7 persons were used as discussants. The discussion was facilitated by principal investigator (PI) of the study and Woreda water resource expert who assisted on guiding, co-facilitating and translating language when needed during the discussion. Three focus group discussions were conducted one in each dietary area. Participants were invited to take part on a voluntary basis and briefed about the study and the possible outcomes for their community. The discussants were maximally stimulated to discuss their opinions freely. The discussions were in the local language (Afan Oromo and Alabegna).

A pre-structured discussion guideline developed by Melaku and Ismail (2002) was modified to include the interventional aspect and used for the discussion. The discussion focused on knowledge and perception of the residents on fluoride, defluoridation, fluorosis and related health problems and dietary habits. In addition, woreda information on future plan in the water sector, annual mean temperature and rain fall were collected. The data were transcribed, restructured and summarized.

4.1.2.3 Household (HH) level information

Information regarding participating HHs and community were collected using structured questionnaire. The HH questionnaire focused on socio-demographic, knowledge, attitude and practices on defluoridation and fluorosis mitigation and dietary habits using Food Frequency

Questionnaire (FFQ) and food security status using Household and Individual Dietary Diversity Score (HDDS & IDDS, respectively).

4.1.2.4 Urine sample collection

Morning urine specimens (≥10mL) were collected in a caped plastic tube from index child and biological mother in each HH. The sample was kept in cooled ice box until brought to laboratory for fluoride and iodide analysis. The ice box was cooled with ice packs which exchanged at 12 hours interval to prevent the samples from decomposition. During night the specimens were refrigerated at nearby health center or hospital. The samples were labeled with unique code. In HHs where there was more than one child, the index child was selected by tossing random number.

4.1.2.5 Dental, Skeletal and Non-skeletal fluorosis assessment

School age children (10-15 years) and biological mother in the selected HHs were assessed for dental fluorosis using Dean Index (Dean, 1934). Subjects were told to thoroughly clean their teeth before the assessment. Skeletal and non-skeletal fluorosis rates were determined using clinical symptoms and physical exercise as developed by Susheela *et al.* (2002) and Shashi and Bhardwaj (2008). According to Susheela *et al* (2002), individuals who could not perform the physical exercise (Figure 2) in the endemic areas were categorized as having skeletal fluorosis.

4.1.3 Phase Three: Mitigation of ingested Fluoride

4.1.3.1 Animal trial

The animal experiment was conducted at Food Science laboratory of Ethiopian Public Health Institute (EPHI). Equivalent age (14 weeks), similar weight (186.7±5.4) and similar sex (all female) of Albino Wistar rats were grouped into four. The rats were kept for 3 weeks until they were well adapted in metabolic cage and to provide washout period as well. The rats were obtained from EPHI zoonoses research section. The supplementation was started in a metabolic cage that enables collection of faeces and urine for 42 consecutive days. Faeces and urine were collected every 24 hrs. The rats were fed on porridge made from *Corn-Soya blend*

(70:30). Leftover food and water was measured every 24 hrs during sample collection. The cages were cleaned every 24 hrs after sampling. The mean daily temperature was 20°C with 12hr day-light cycle.

Diet

The calcium or moringa given for the treatment groups were blended with in-house prepared ration (CornSoya blend, CSB – 70:30), assuming the amount that can combat fluoride available in water. The water was fluoridated at 10 mg/L. Calcium used for supplementation was used as calcium citrate (500mg, Solgar LTD, USA) and moringa dry leaf (*Moringa Stenopetala*) which was used in the trial were collected from Konso, SNNPR, Ethiopia. The fresh leaf was collected and dried at room temperature in EPHI laboratory. The categories of rat, ration and supplementation provided during the study period are summarized in table 4.

Table 4: Summary of ration and supplements provided for mitigation of ingested fluoride

Category	Group 1	Group 2	Group 3	Group 4:
Group code	FF	FC	CA	M
Water	Non-fluoridated	Fluoridated water at 10ppm	Fluoridated water at 10ppm	Fluoridated water at 10ppm
Ration	Corn-Soya blend (CSB: 70: 30)	Corn-Soya blend (CSB: 70: 30)	Corn-Soya blend (CSB: 70: 30)	Corn-Soya blend (CSB: 70: 30)
Supplements	None	None	Calcium tablet* blended with CSB (0.5mg tablet/15g CSB)	Moringa Stenopetala leaf* powder blended with CSB (0.1g dry leaf / 15g CSB)

*0.2mg Calcium equivalent

4.1.3.2 Human trial

The human trial was conducted at Halaku/Adamitulu for eight days including the first day which was used as a baseline. The subjects were recruited from Halaku community based on set inclusion criteria. The inclusion criteria of the subjects were being in reproductive age,

non-pregnant, non-lactating and being in good health (have no any informed sickness). Twenty eight subjects who fulfilled the inclusion criteria were selected randomly from willing women (N = 96) and categorized and assigned randomly into one of the four groups namely; Milk, Moringa, Calcium and Control. Each group was comprised of seven subjects. Moringa and Calcium group were given 5kg wheat flour which was blended with dry moringa leaf or calcium citrate (both 200mg calcium equivalent). The milk group was given 250 mL milk daily with wheat flour. The control group were given wheat flour alone. All groups were instructed to bake and prepare bread (150g flour). A standard measuring apparatus was given to subjects for measuring the flour. The bread was eaten at dinner time after which they drink water as usual. The milk group was instructed to drink the milk (before dinner). All the study participants were taking the foods under supervision of health extension workers. They were instructed not to share the foods they prepared and to bring back any left over. However, they were allowed to prepare separate meal for family in case they need to do so. The study participants were instructed to take their usual consumption of food, beverage and water in addition to the supplement food. Each day the subjects collect morning urine in a coded plastic tube and delivered to the health extension workers. The health extension workers checked for consistency, volume and code. Urine samples were quickly stored in ice box cooled with ice puck until brought to laboratory.

4.2 Ethical clearance

The project was approved and ethical clearance was obtained from Ethiopian Public Health Institute (EPHI). Letter of support was also obtained from regional health offices. The study was conducted according to rules and guidelines of Research and Ethical Clearance committee (RECC) of Ethiopian Public Health Institute (EPHI). Verbal informed consent was obtained after the purpose and methods of the study had been fully explained to head of selected households. Woreda (district) health, Kebele (subdistrict) administrators and health extension workers including woreda water supply experts were briefed the objective of the study. The HHs were requested for willingness to provide morning urine, prepare duplicate plate, and to keep the usual diet habits of the participants and to duplicate the diet as precisely as possible by observing the amounts consumed by the subjects. The households who

participated on duplicate plate preparation were compensated by money equivalent to food and food ingredients collected for each subject and time wasted for responding to questionnaire. Any clinical cases observed during the survey were advised to go to nearest health institution for treatment.

Prior to the clinical trial, informed consent was obtained from the women to participate in the study. The study participants were informed about the potential benefit of supplementation and/ or diversifying diet with calcium rich sources. They were told the right to withdraw from the study at any stage.

4.3 Laboratory Apparatus and Reagents

4.3.1 Apparatus and materials

The laboratory apparatus and materials used for the study included: refrigerators (-20, -45 and -4°C, Thermo scientific, UK), freeze drier (CHRiST BETA 1-8 LD plus, SciQuip ltd,UK), nickel crucibles, analytical balance (160 ± 0.0001 g) (Ainsworth AA-160, Diver Instrument ltd, USA)), spatula, pipettes, pipette fillers, hot plate, muffle furnace (Carbolite, UK), goggle, tongs, oven, washing bottles, 50 mL plastic beakers, magnetic stirrer, magnetic road, 50 mL graduated plastic tubes, filter funnels, filter papers, different sizes of volumetric flasks, plastic beakers (50mL), distiller (Aquatron, Bibby Scientific ltd, UK), de-ionizer (Barnstead E-pure, Thermoscientific, USA), pH/ISE meter (Orion Model, EA 940 Expandable Ion Analyzer) equipped with combination fluoride-selective electrode (Orion Model 96-09; Orion research, USA), dual channel Jenway pH/Ion meter, PerfectION (Mettler Toledo, Germany) fluoride ISE, pH glass electrode and BNC connector(Mettler Toledo, Germany).

4.3.2 Chemicals and Reagents

NaOH pellets (ACS grade), HCl (37% sp.gr. 1.19), buffer solutions for pH calibration, NaCl (99%), EDTA, tri-sodium acetate, glacial acetic acid, 2 bottles of 1000ppm fluoride calibration standard as Sodium fluoride, 2 bottle of TISAB III and II and NaF (ACS grade). All solutions were prepared with double distilled and/ or de-ionized water.

4.3.3 Food items used for clinica trial

Moringa dry leaf powder, Calcium Citrate (USA), Milk (MAMA milk, Sebeta Agroindustry, Ethiopia), Corn-Soya blend (70:30%, mature seed, toasted and ground in house, EPHI experimental kitchen) and Wheat flour (Kality foods Share company, Ethiopia).

4.4 Sample Pretreatment and Laboratory Analysis

4.4.1 Sample pretreatment and moisture content determination

The pooled duplicate samples (food sampled over 24 hours) collected were ground and homogenized using blender (Philips, Hungary).

4.4.1.1 Moisture content determination

Hundred gram fresh samples were taken and lyophilized to constant weight using freeze drier (CHRiST BETA 1-8 LD plus, SciQuip ltd, UK). The moisture content was determined from the difference between fresh sample and dried weight. The percent moisture were calculated following the AOAC method (AOAC, 2000: 925.09)

Calculation: % Moisture $= (W/W_1) \times 100$

Where: W_1 = Weight of fresh sample

W_2 = Weight of dry sample

W (weight lost) = W_1-W_2

4.4.2 Analytical Procedures for sample preparation for fluoride analysis

The fluoride level in food samples were determined based on the alkali fusion and fluoride ion selective electrode method (Malde *et al.*, 2001). Urine samples (both human and rat) were directly determined by adding TISAB III based on the method of Jenway (Jenway, 2011). The rat feces were treated similar to food samples. Spiked food, feces and urine samples were analyzed using known addition methods (Jenway, 2011).

4.4.2.1 TISAB (III) Preparation

In addition to purchased one from Metler Toledo (Germany) TISAB (III) was prepared by dissolving 58 g sodium chloride, 2 g EDTA, 7 g tri-sodium acetate and 57 mL glacial acetic acid in 500 mL deionized water as per the company mannual (Jenway, 2011). Finally the pH

was adjusted to 5.2 by using 5 M sodium hydroxide solutions and volume adjusted to one liter with deionized water.

4.4.2.2 Standard Preparation

Standards were prepared by series dilution of the (stock) standard (1000ppm F) purchased with the electrode (Mettler Toledo). Consecutive dilutions were used to prepare standards at 100, 50, 25, 20, 10, 5, 0.5 and 0.05 ppm working standards. The standards were used depending on expected fluoride level of the samples.

4.4.2.3 Calibration of the electrode

After the ash had settled and the solution was clear, aliquots of 10 or 5mL sample were taken out and mixed with equal amount of TISAB III solution. TISAB was added to obtain a pH of 5.2-5.4, which is the optimum pH range for fluoride determination. The same amount of TISAB was also added in to equal amount of deionized water (blank) and in each 10 mL of standard solutions. Then, the instrument was calibrated using blank and standard solutions of fluoride concentration; 0.05, 0.5, 5, 10 and 20 mg/L.

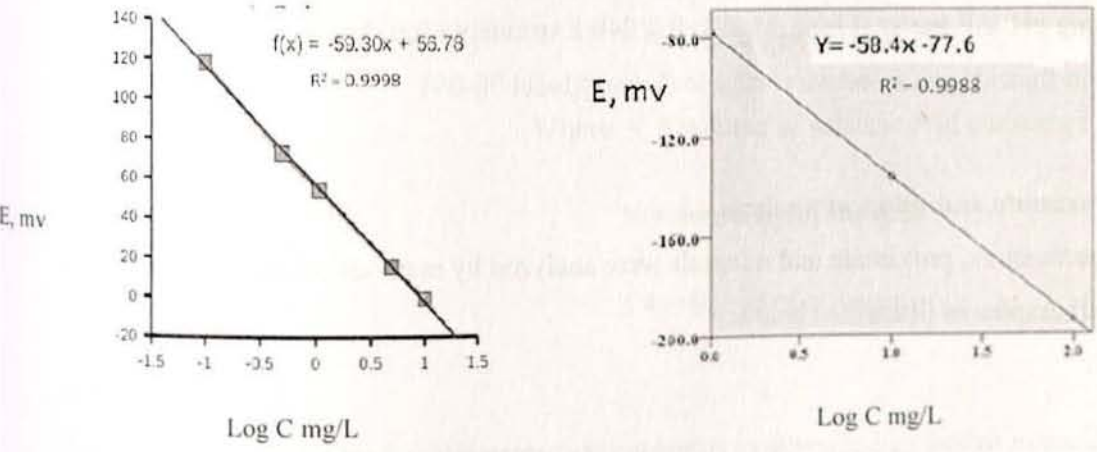

Figure 11: Calibration curve for fluoride electrode

4.5 Calibration Curve

The standard curve (Figure 11) of fluoride reflects the ratio between the content of fluoride in a solution and the resulting measurement response. In preparing a standard curve, the following concentrations were used: 0.05, 0.5, 5, 25 and 50 mg F-/L. The calibration curve shows a linear relationship between fluoride concentration in the standards and -mV-reading. This is in agreement with the Jenway working manual (Jenway, 2011).

4.5.1 Fluoride analysis

Urinary fluoride, food beverage feces and water were analyzed using PerfectION™ combination fluoride ion selective electrode (Mettler Toledo, Germany) coupled with bench top dual channel ion-meter (Jenway, model 3345, England) at Ethiopian Public Health Institute (EPHI). The samples were determined potentiometrically using a fluoride ion-selective electrode after calibrating the electrode with 5 point calibration standards. The methods used were that of Orion (Orion, 1991) for urine and water and method of Malde *et al* (2001) for food, beverage and feces. Since the matrix of feces is similar with that of food except indigestible polysaccharides which would be decomposed during ashing, the fluoride in feces was determined using the method adapted for food by Malde (2001). Fluoride both in water and food was partially determined at chemistry department, Addis Ababa University (AAU) using pH/ISE meter (Orion Model, EA 940 Expandable Ion Analyzer) equipped with combination fluoride-ion selective electrode (Orion Model 96-09).

4.5.2 Proximate and mineral analysis

All other parameters; proximate and minerals were analyzed by methods of AOAC from dried and ground samples as illustrated below.

4.5.2.1 Total Ash

The total ash content of food samples were determined following the procedure of AOAC (2000) and calculated as % ash as follows:

Calculation: % Ash $= (W_2/W_1) \times 100$

Where: W_1 = Weight of sample (dry weight base)

W_2 = Weight of ash

4.5.2.2 Crude Protein

Kjeldahl method was applied to determine nitrogen content and therefore protein determination (AOAC, 2000). The crude protein value was calculated from total nitrogen using the factor of 6.25 as per Foss tecator manual (Tecator, 2003)

Calculation: mg nitrogen in the sample $= V \times N \times 14$

g nitrogen / 100 g sample = mg of nitrogen x 100/ mg sample

Total nitrogen (%) $= (V^b \times N \times 1.4)/ W$

Crude protein (%) = total nitrogen (%) x 6.25^a

Where: V = volume of sulfuric acid consumed

N = normality of the acid

1.4 = Eq. wt of nitrogen

4.5.2.3 Crude Fat

Fats were determined by Soxhlet extraction apparatus as described in tecator manual (Tecator, 1998). The amount of fat was quantified gravimetrically and calculated from the difference in weight of the extraction flask before and after extraction as percentage of crude fat (AOAC, 2000).

Calculation: $W = W_2 - W_1$

Fat g/ 100g fresh sample = W x (100 - % moisture) / W_D

Where: W = weight of fat

W_2 = weight of extraction flask after extraction

W_1 = weight of extraction flask before extraction

W_D = weight of dried sample

4.5.2.4 Crude Fiber

Crude fiber was determined following the procedure of AOAC (2000). The undigested residue collected after digestion was ignited and loss in weight after ignition calculated as follows:

Calculation: Crude fiber g/100g = $(W_1-W_2)(100 - M) / W_3$

Where: W_1 = Crucible weight after drying

W_2 = Crucible weight after drying

W_3 = Sample dry weigh

M = % moisture content of the sample

4.5.2.5 Carbohydrate

Total carbohydrate content of food samples (per 100 g) were calculated by difference using the proximate composition as follows:

Total carbohydrate = 100 − (Crude protein + Crude fat + Ash + Moisture content)

4.5.2.6 Minerals

The mineral contents of water samples were determined using atomic absorption spectrophotometer after acidifying as per the instruction manual of the company. The mineral contents of food samples were determined from the ash product as per the AOAC method (2000). Calibrations of the instrument was conducted using the instruction manual of the manufacturer (Shimadzu, 2012)

4.5.3 Quality control

Each sample; food, water, beverage, urine (both human and rat) and feces (rat) was analyzed in duplicates. All the analysis; proximate, minerals and fluoride were analyzed against blank samples and along with standards for quality control. More over Certified Reference Materials (CRM) were also analyzed along the samples to check the accuracy and sensitivity of the method. Plant tissue made by National Institute of Standards and Technology (NIST, USA) and fish tissue prepared in house by National Institute of Nutrition and Seafood Research (NIFS, Norway) was used to validate the method for fluoride analysis.

4.5.4 Data analysis

Data obtained from the assessment were intered and using analyzed using spread sheet (Window 7) and SPSS volume 20. Fluoride content and nutrient level of foods were calculated for mean intake, percent of recommended daily allowance and upper tolerable intake. The clinical trial data was calculated for comparison of mean and significannt difference between treatment groups and control using non-parametric analysis.

CHAPTER FIVE: RESULTS AND DISCUSSION

5.1 Fluoride sources

More than 93% (N = 48) of drinking water samples collected from Ethiopian rift valley contained fluoride concentration above WHO guideline (WHO, 2008) for drinking water. About 80% of the water samples (Figure 12) were in the range that can cause skeletal fluorosis.

Assuming the low altitudes of the Rift Valley (<1500 above sea level) and annual high mean temperature, which induces extra water intake, it is clear that the population in the rift system is under critical fluoride contamination.

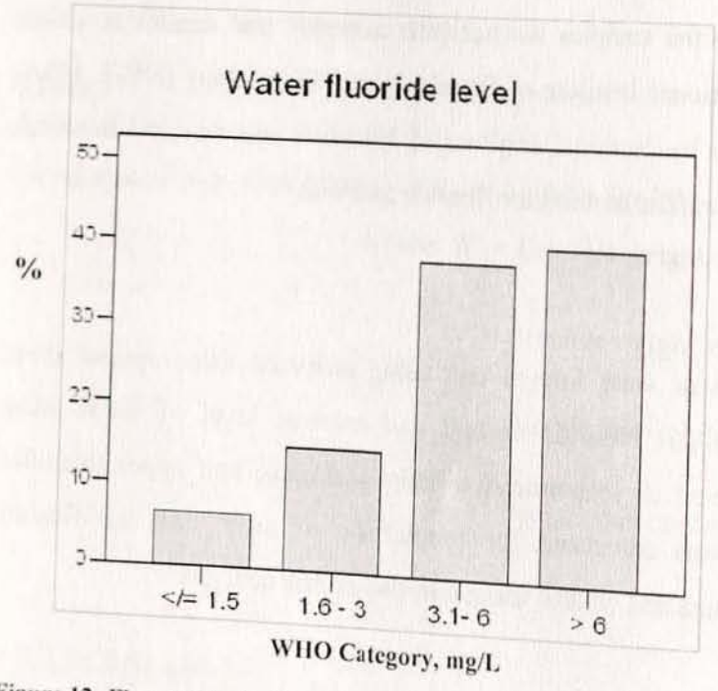

Figure 12: Fluoride level of water sources of ERV categorized based on WHO Cut-off points

Brouwer and his colleagues (1988), reported that fluorosis can occur at fluoride concentration as low as 0.8mg/l depending on water intake and dietary habits. But fluoride concentration in the study areas were much higher than (Table 5) the guideline value of 1.5mg/L set by World Health Organization (2008).

The fluoride content of different food ingredients collected from rift areas is tabulated (Table

6). The samples were collected from HHs as well as community market, during market days. The results show that Teff and chili powder had the highest amount of fluoride. Rape seed, flax seed, wheat and maize flour bought from the local market have significant amount. It is clear that if these food ingredients are prepared using fluoride contaminated water, it will have fluoride content that exceeds above the upper tolerable level. Finger millet, fish tissue, "Shiro" (beans powder), tomato and onion are among the food ingredients containing appreciable amount of fluoride. Moreover food items such as rice which has water absorption capacity may accumulate more fluoride than others (Tegegne *et al.*, 2013).

Table 5: Summary of fluoride and minerals content of potable water (mg/L), (Mean ± SD)

Region	Fluoride	Iron	Zinc	Calcium	Phosphorus
Northern ERV Afar and kereyu (N =11)	4.1 ± 1.7	0.3 ± 0.4	0.1 ± 0.0	1.6 ± 1.6	0.5 ± 0.2
Central ERV Oromia (N = 22)	8.7 ± 2.7	0.1 ± 0.0	0.1 ± 0.0	1.7 ± 0.5	0.4 ± 0.1
Southern ERV, SNNPR N = 15	4.3 ± 1.5	0.3 ± 0.2	0.1 ± 0.0	2.0 ± 0.9	0.4 ± 0.1

The fluoride content of selected staple foods varied from 1.3-3.2 mg F/kg (Table 7). Sauce was not collected in this study. From water and recipes presented here it is clear that daily fluoride intake from water alone excluding intake from food, will exceed the daily recommendation if calculated per person per day. In this study staple foods made of millet and Enset (false banana) show high concentration of calcium.

Table 6: Fluoride content of food ingredients*

Food type	N	Mean, mg/kg	Stdev
Cereals			
Barley	1	5.0	-
Maize flour	5	7.2	1.3
Maize, white	5	4.0	0.7
Wheat, grain	1	4.0	-
Wheat flour	2	7.2	0.7
Sorghum, red	2	4.2	1.1
Finger millet, brown	3	6.0	3.0
Teff, mixed (red-white)	5	14.8	5.0
Oil seeds			
Rapeseed, brown	1	9.0	-
Flaxseed, red	1	9.0	-
Animal products			-
Milk (mg/L)	3	0.4	0.1
Fish tissue, tilapia	2	5.4	1.1

Food type	N	Mean, mg/kg	Stdev
Legumes			
Beans powder, Shiro	4	6.3	0.3
Kidney bean	2	4.1	0.1
Vegetables			-
Pepper, Green	6	5.0	1.7
Potato	10	5.0	1.2
Kale	10	4.9	1.0
Sweet potato, orange flesh	1	3.0	-
Tomato	3	5.9	1.5
Garlic	1	4.2	-
Onion	5	6.1	0.4
Others			
Khat (*Catha edulis), leaf*	1	5.0	-
Moringa, dry leaf	1	6.0	-
Chilli powder	4	17.3	4.2
Moringa 4% in CSB	1	6.0	-
Corn-Soya blend 70-30	1	7.0	-

*Dry weight base, all raw

Table 7: Fluoride, minerals and proximate composition of staple foods from ERV (Mean±/ Stdev)

Food Type*	F- water (mg/L)	Fluoride (mg/Kg)	% Moisture	% Ash	% Fat	% Protein	% Fiber	Ca food	P food	Fe food	Zn food
Bread, wheat(n=7)	5.9±3.9	1.4± 0.3	34.1±3.2	2.3±0.6	0.6±0.1	7.5±0.7	3.2±2.0	50.0±8.3	15.7±5.8	5.1±2.8	1.1±0.1
Injera, maize (n=9)	4.6±2.3	2.4± 2.1	57.7±6.2	1.7±0.1	1.2±0.4	4.2±0.6	4.3±1.6	40.6±14.6	50.2±15.0	9.5±2.0	2.6±0.2
Injera, teff (n=13)	3.7±1.4	1.8± 0.4	59.2±6.4	2.2±0.3	1.0±0.5	4.6±0.7	7.2±1.9	57.0±17.9	64.9±61.4	24.6±14.5	2.9±0.6
Bread Local, maize, (n=14)	3.3±1.0	1.3± 0.2	50.3±3.3	1.7±0.2	1.6±0.3	5.2±0.5	4.0±1.4	39.0±11.1	67.8±40.5	8.2±1.8	2.5±0.2
Bread Local, wheat (n=7)	5.4±2.9	3.2± 2.0	45.7±2.7	2.1±0.3	0.9±0.3	7.1±0.6	5.2±1.4	32.4±8.6	35.0±7.5	7.7±2.9	3.1±0.9
Unleavened bread ,millet(n=3)	5.5±1.6	1.8± 0.3	45.7±4.7	2.2±0.1	2.4±0.5	5.1±0.7	7.6±5.4	245.2±25.6	60.6±18.0	6.4±1.9	1.9±1.1
Unleavened bread ,maize(n=12)	4.3±1.6	2.0± 0.9	41.3±2.6	2.1±0.7	2.0±0.3	5.7±0.4	5.1±1.0	38.5±12.6	45.6±20.5	7.4±2.6	2.4±0.2
Unleavened bread, maize + Enset (false banana) (n=1)	9.5	1.5	42.8	1.1	2.6	5.1	5.4	52.4	349.9	3.3	2.6

*dry weight base

McGown and his colleagues (1976) in their work on animal trial and described that dietary fat increases fluoride absorption by increasing gastric retention time as observed in increase of urinary fluoride and decrease of feces fluoride.

Tea leaf has high level of fluoride per gram of dry leaf. As can be observed from the table (Table 8) tea leaf sold in Ethiopian market contain significant amount of fluoride. Entirely all tea leaves have high fluoride content and the level increases when boiled in fluoride contaminated water (Table 8). Malde et al (2006) reported that tea leaves may absorb or release fluoride depending on fluoride level in water used to brew the tea. In this analysis we did not observe such a property. One explanation for this could be because the water used for brewing the tea in our case has similar fluoride level with the tea leaf (≈ 5mg F^-/L). Therefore community who use fluoride contaminated water should not use tea as a beverage or use fluoride free water to brew it in order to reduce fluorosis risk. Consumption of tea is one of the risk factors for fluoride contamination and fluorosis (Zerabruk *et al.*, 2010).

Table 8: Fluoride content of beverages: tea brewed with or without fluoride contaminated water

2.5g tea leaf (brand name)boiled in 100ml water	mgF/L in tap water (F^- = 0.14) mg/L)	mgF/L in 5 mg/L fluoridated water	Recovery (%)
Wushwush	6.0	12.7	104
Anbessa	4.4	9.6	104
Gumero	4.9	10.3	108
Abysinia	4.2	9.8	112
Addis	5.5	11.0	110
Good morning	7.1	12.2	102
Kerefa (cinnamon)	0.2	5.7	110
Ginger	0.3	5.6	106
Tosigne (thymol)	0.3	5.2	98
Coffee	0.2	5.7	110

5.2 Knowledge and source of information about fluoride and fluorosis

All the discussants mentioned that the cause of the mottled and brown teeth and skeletal problems is the water which they believe to contain "bad mineral". None of the discussants mention the word fluoride.

Health extension workers and health development armies have been giving health education at health post level. The topics of the teaching were points outlined in health extension program like sanitation, tuberculosis, vaccination, birth control, malaria, and so on. But most of the discussants attest that education on fluoride related problems has never been raised. They mentioned that it probably was not a point of attention because they might have thought there is nothing to do about it. Almost none of the discussants in all the areas mentioned had been actively taught about fluoride related problems at all, especially by health workers.

Health extension workers in Halaku and Benti mentioned that the water cause no significant health problem although the taste of the water is not plausible. They also use same water when they work the whole day in the community. They didn't understand or have no information about the cumulative effect of consuming fluoride contaminated water. Most people don't consider the effect of fluoride beyond the cosmetic effect on teeth.

5.3 Perceived health, social and economic consequences due to fluoride

Most of the focus group participants did not give emphasis on teeth discoloration but according to the discussants, adolescents feel that they were singled out when going to other areas and girls particularly felt a bit ashamed of having such discolored teeth. They often had to cover their mouth while laughing. Some often mention their teeth smell foul, very fragile and painful when they eat hard foods. Older people have fear of bowing down and stiffness of the back, neck and the joints, which interferes with their day to day activities. The communities in all the three areas are suspicious about the future of staying in the area unless the water source is changed or something is done by the government. The male discussants have fear of bowing down of the back bone, being less efficient in day to day activity including sexual relationship. Some even further explain that their cattle are also in a serious

health problems because of the excessive level of the chemical.

The community associated most of the health problems particularly dental and skeletal ones with the water they get from borehole. According to the discussants all people in the communities are aware of the existence of dental and skeletal problems arising due to drinking water from the borehole. Generally, across all groups it was felt that the treated pipe water was very safe for consumption. Hence, they would prefer to drink water from such treated source. Communities in Benti (in In Fentale) expects the government to change the water source to Metahara and Halaku community expects to get piped water from Adamitulu town. But the water source in Adamitulu town also contains fluoride level above WHO recommendation.

The teeth problems were reported to start at early ages and are highly prevalent. Yet, it is not commonly perceived as a major problem because of the wide spread prevalence. Their only concern was that in those severely affected, the teeth were weak and fragile and this creates difficulty in chewing hard food such as "Kolo" - toasted grain, "Kitta" -hard bread and sugar cane. Discussants in Halaku further told that it's so distressing not be able to chew Kolo and Kitta. The inhabitants feel as they miss something which people in other area can do it. They also ask back why some members of the households didn't get mottled teeth even though they are born and brought up in the community. In some families some of them have completely white teeth while others have mottled teeth like the rest of the people in the localities. Why teeth of these persons remained white is difficult to explain.

Case study: Zeneba is a girl of 11 years old living in Halaku, Adamitulu woreda. Although her brothers and sisters have mottled teeth, hers is completely white. We learnt from her parents that at the age between three and five, she lived with her ants in the nearby Woreda.

5.4 Food taboo and dietary habit:

In all the three dietary areas, the communities are Muslims. There are no as such foods forbidden, unless the economical status and availability of food items on local market limit dietary habits. In all the three areas maize, coffee, legumes and milk are common. Among all

the studied communities some food like milk is shared with neighbors. Consumption of milk in Alaba and Adamitulu is limited because of absence of cattle in most of the households. In Fentale, all sort of cattle including camel and goat are available and consumption of milk is common. The consumption of vegetable and fruit is almost none except onion and potato. Of course kale was commonly consumed in Alaba and Adamitulu. Food is prepared in metallic pan in almost all households. Water is kept in a plastic (pot); "Jerican".

5.5 Trend of harvesting rain water:

People prefer rain water rather than water from the bore hole due to better taste. According to the discussants, rain water is the safest water if harvested and handled hygienically. Good facilities are needed in order to collect in safe way. In Alaba and Fentale there is a trend of harvesting rain water. In Fentale, water is collected from a pond made by the flood. In Alaba people usually harvest rain water from roof of a house. This trend is encouraging if used to tackle fluoride contamination.

5.6 Suggestions and willingness to participate in preventive measures:

The Fentale and Halaku community have a chance of getting water from Awash and Ziway Lake respectively. The communities in Qobochobare have no such option, although Bilate River originates in the area, it is seasonal. All the discussants have expressed willingness to participate in any public health activity intended to provide the community with a safe water supply on a continuous basis.

5.7 Knowledge Attitude and Practices (KAP) of the community

Even though people failed to mention the word "fluoride" as a cause of fluorosis, they had a good under-standing that the problem is related to the consumption of water containing "bad minerals". The dental fluorosis is mainly accepted as a cosmetic and only given emphasis by adolescents. Some who are severely affected by dental fluorosis are also concerned because of the pain and inability to chew hard foods. Adults also have realized that restricted mobility and flexibility in back bone, neck and for arms, which made them unable to perform their day

to day activities. As observed during the discussion the male seem to be more affected than their female counterpart. Some of the discussants have a concern regarding the economic consequences due to skeletal fluorosis and parallel effect of fluoride on children, particularly on mental activity.

In this study, dental fluorosis was not considered as a major and priority health problem by most of the groups including health extension workers. This may be due to the progressive nature of the medical problem and the fact that often it is painless and non-life threatening as well as due to the fact that it affects the majority of people.

According to the focus group discussants in the three study areas there is no dietary restriction and taboo than written in Islam books. People do not have information on dietary risk factors for fluorosis or any food that might help to mitigate ingested fluoride. In Alaba the communities have information on defluoridation technologies due to its implementation in nearby Kebeles (smallest administrative unit in the government structure). The people in Fentale (Kereyu) are much concerned on cleaning teeth by a stick made from selected plants to prevent teeth discoloration.

Lack of knowledge concerning fluoride and its health consequences in women deserves due attention and action. Since most of these women are in child-bearing age, educating them would have a great impact on the success of future intervention programs. Therefore fluoride contamination and mitigatory measures should be incorporated into the routine health extension workers program package. Another concern coming out of this study is that health workers seem to have avoided teaching about fluoride contamination and fluorosis and measures to be taken for mitigation. Many respondents said that they were told nothing about fluorosis from professionals. This could be because of absence of guideline and singled out mitigation measures given by regional as well as Federal Ministry of Health.

The effect of fluoride contamination and its mitigation should get sufficient attention by the health workers and appropriate health education should be given. Moreover further study should be conducted on defluoridation technologies, dietary risk factors and mitigatory

techniques. Other studies in Africa have shown that the more information people get about dental problems from health workers and the media, the more those informational media were trusted, the higher their personal risk appraisal for their dental problems (Astrom *et al.*, 1999).In India, researchers have become successful in mitigating ingested fluoride and in reversing skeletal fluorosis at young age (Susheela and Bhatnagar 2002).

The economic consequences to persons affected by skeletal fluorosis should also be of concern. From the discussions of the study one can learn that those who have lived long enough in the locality have already developed some kind of skeletal fluorosis. The consequences of fluorosis on health, productivity of the households at particular and its economic impact at national in general should not be underestimated. The study showed willingness of the community to participate by all the groups in activities directed at improving the provision of safe water supply to the community. Health and nutrition education will help to reduce the effect of ingested fluoride until either defluoridation techniques are employed or the water source is changed.

5.8 Fluoride intake and dietary sources

As can be seen from table 10, the dietary fluoride contribution of food in Alaba and Adamitulu is higher than that of water while in Fentale the contribution of water is higher than in food. Basically foods that have high water absorption capacity like rice (Tegene *et al.*, 2013) accumulate high amount of fluoride. The total dietary fluoride intakes of children as well mothers are above the upper tolerable limit of 10mg/day except in Adamitulu.

5.9 Fluoride and nutrient dietary intake

Although the Fentale community drank coffee with more than 50% volume of milk which they call it "Oja" the fluoride contribution of Oja is very high compared to cereal based areas (Alaba and Adamitulu). From the mean comparison it is clear that there is significant difference between Adamitulu and the rest (Alaba and Fentale) ($P<0.005$). The fluoride

content in the dietary sources for both children and mothers have similar trends in all the study areas. According to EU standard for fluoride intake, the amount of fluoride ingested by children is above the upper tolerable limit of 0.1mg/bw/day (EFSA, 2006).

Table 9: Amount of food, beverage and water consumed: children & mothers

Subjects	Diets consumed	Woreda	N	Mean	Stdev.	Min- Max
Child	Food, gm	Fentale	20	363	82	187 – 509
		Alaba	20	905	269	522 -1527
		Adamitulu	20	498	183	232 – 879
	Coffee, ml	Fentale	20	550	222	180 – 1100
		Alaba	20	232	112	57 – 495
		Adamitulu	20	151	49	72 – 272
	Water, ml	Fentale	20	938	443	123 – 2000
		Alaba	20	735	277	400 – 1500
		Adamitulu	20	530	177	380 -1040
Mother	Food by gm	Fentale	20	3835	98	182-562
		Alaba	20	977	309	618-793
		AdamiT	20	572	208	299- 1091
	Coffee or Oja, mL	Fentale	20	974	345	480-1650
		Alaba	20	331	140	130-622
		AdamiT	20	171	58	90-308
	Water , mL	Fentale	20	1139	522	230-1950
		Alaba	20	1004	271	500-1500
		AdamiT	20	618	269	400-1560

Mean food consumption per gram is higher in Alaba than Fentale and Adamitulu. But water and coffee consumption volume is higher for Fentale than the other two districts (Table 9). Generally, as the temperature in Fentale is high the fluid consumption is indicative. Coffee with large proportion of milk is taken in Fentale as a substitute for water. This might help to reduce ingested fluoride and fluorosis by reducing intake of fluoride contaminated water. This assessment was conducted in summer, when the temperature in study areas is below the annual average. The consumption of both water and beverage might be higher in hotter season.

Iron intake (mean) is above Upper Tolerable level (UL) in Alaba and Adamitulu (Table 11). Calcium and Zinc are the most limiting nutrients in Fentale (below RDA) (Whitney and Rolfes, 2005). The dietary intake of calcium from milk was not included in the current

calculation. The mean daily phosphorus intake in Fentale is almost half of RDA while that of Alaba is above RDA but below the Upper Tolerable Level (UL).

Table 10: Dietary fluoride intake (mean, N = 20 in each area) and its sources

Fluoride sources	Child			Mother		
	Fentale	Alaba	Adamitulu	Fentale	Alaba	Adamitulu
Food	2.4	7.9	4.4	2.5	8.6	5.0
Water	6.5	4.6	2.6	7.9	6.2	3.0
Beverage (coffee)	2.3*	0.4	0.8	4.3	0.7	0.9
Total	11.2	12.9	7.8	14.7	15.5	8.9
DI in *mgF/bw/day*	0.43 [a]	0.44[a]	0.25[b]	0.29[c]	0.29[c]	0.19[d]

C** = Child; M** = mother; *Beverage consumed is entirely coffee except in Fentale with added milk; Values with different superscripts are significantly different.

5.9.1 Mineral intake

Table 11: Mineral intake of children and mothers (mg/day) from food

Nutrients (mg/day)	Woreda	N	Mean (Stdev)		Children (9-13)		Mothers (adult)	
			Child	Mother	RDA	UL	RDA	UL
Iron	Fentale	20	35.1 (13.7)	35.9 (11.6)	8	40	8	45
	Alaba	20	245.6 (133.6)	267.2 147.4)				
	AdamiT	20	40.8 (22.1)	46.6 25.1)				
Zinc	Fentale	20	6.9 (1.9)	7.3 (2.3)	8	23	8	40
	Alaba	20	20.3 (8.1)	21.8 (9.3)				
	AdamiT	20	10.8 (4.0)	12.4 (4.4)				
Calcium	Fentale	20	94.0 (80.4)*	95.2 (72.9)	1300	4000	700	4000
	Alaba	20	1862.0 (981.0)	2013.2 (1133.9)				
	AdamiT	20	375.8 (344.6)	448.5 (459.3)				
Phosphorus	Fentale	20	787.0 (287.3)	817.9 (274.1)	1250	4000	700	4000
	Alaba	20	2057.2 (966.7)	2195.6 (1047.6)				
	AdamiT	20	1490.8 (548.5)	1699.9 (586.0)				

*calcium content from milk not included

5.9.2 Daily fluoride intake category

Table 12: Daily fluoride intake above and below tolerable level in the selected woredas

mgF per day	Category (mg)	Fentale	Alaba	Adamitulu
Child	0-2 (Adequate intake level)	0.0[a]	0.0[a]	0.0[a]
	2.1-10 (Tolerable level)	45.0[a]	40.0[a]	75.0[b]
	> 10 (Above UL)	55.0[a]	60.0[a]	25.0[b]
Mother	0-4 (Adequate intake level)	0.0[a]	0.0[a]	0.0[a]
	4.01-10 (Tolerable level)	10.0[a]	15.0[a]	70.0[b]
	above 10 (Above UL)	90.0[a]	85.0[a]	30.0[b]

Values with different superscripts are significantly different

Table 13: Fluoride intake per body weight at 60 & 100% bioavailability and urinary fluoride level

Subjects	Fluoride intake by Children and biological mothers	Study sites	N	Mean	Stdev	Range
Children	mgF / day	Fentale	20	10.9	3.1	5.8-16.2
		Alaba	20	12.9	4.9	7.5-24.3
		Adamitulu	20	7.7	2.7	4.4-14.3
	mgF / day / bw at 100% bioavailability	Fentale	20	0.4	0.1	0.3-0.8
		Alaba	20	0.4	0.2	0.1-1.2
		Adamitulu	20	0.3	0.1	0.1-0.4
	mgF / day / bw at 60% bioavailability	Fentale	20	0.3	0.1	0.0-0.4
		Alaba	20	0.2	0.1	0.1-0.3
		Adamitulu	20	0.1	0.0	0.1-0.2
	Urinary fluoride, mg/L	Fentale	20	8.4	5.8	1.9 - 20.5
		Alaba	20	3.3	2.2	0.5 - 9.1
		Adamitulu	20	9.0	4.5	2.4 - 19.0
Mother	mgF / Day	Fentale	20	14.7	3.3	9.9-23.8
		Alaba	20	15.6	6.3	8.8-31.5
		AdamiT	20	9.0	2.9	5.8-14.9
	mgF / BW at 60%F Bioavailability	Fentale	20	0.2	0.0	0.1-0.3
		Alaba	20	0.2	0.1	0.1-0.4
		AdamiT	20	0.1	0.0	0.1-0.2
	mgF / bw at 100% F Bioavailability	Fentale	20	0.3	0.1	0.2-0.5
		Alaba	20	0.3	0.1	0.1-0.7
		AdamiT	20	0.2	0.1	0.1-0.4
	Urinary fluoride, mg/L	Fentale	20	7.4	6.0	0.3-21.4
		Alaba	20	4.8	3.7	1.0-15.5
		AdamiT	20	11.0	7.2	1.7-23.7

Fluoride intake in all the three selected dietary areas is above adequate level in children (Table 12). The intake above the tolerable level is above 55% in Fentale and Alaba while it is 25% in Adamitulu children. Although the daily intake level is lower than the other two districts, the fluorosis rate is higher in Adamitulu. The intake of fluoride in mothers in Fentale and Alaba are above 85%. For both children and mothers fluoride intake above tolerable level is less than 30% in Adamitulu.

According to Extrand and Ehmebo (1979), fluoride absorption is 100% on fasting stomach and 60% with Ca-rich meal. As shown in table 13, it is attempted whether the fluoride intake higher or lower than the Low Observed Adverse Effect Level (LOAEL) of 0.1mg/bw/day at different bioavailability. From table 13 it is seen that the fluoride intake at 60% bioavailability is above the LOAEL in all the woredas except for Adamitulu. Assuming high mean temperature in the rift valley, the fluoride ingestion might even be higher than observed in this assessment. This study is conducted in summer season when the temperature is relatively low and intermittent rain fall exist, which would lower fluid intake.

Table 14: Risk assessment of ingested fluoride based on daily F intake

Parameter	Child	Fentale		Alaba		Adamitulu	
		Child	Mother	Child	Mother	Child	Mother
DI	At 100% BA	0.42	0.29	0.44	0.29	0.25	0.19
	At 60% BA	0.25	0.17	0.26	0.18	0.15	0.11
HQ	At 100% BA	8.38	5.82	8.76	5.88	4.94	3.80
	At 60% BA	5.03	3.49	5.26	3.53	2.96	2.28
GV	At 100% BA	0.45	0.61	0.71	0.98	1.17	1.15

From EU standard (EFSA, 2008)

TDI = 0.05 LOAEL = 0.1 NOAEL = 0.05 UF = 1.0 UL = 0.1

Children, 9-13 yrs (Mother): AI = 2 (3) mg/day UL = 10mg/day (same for both age)

Although urinary fluoride is used as a biomarker for ingested and circulatory fluoride, the level depends on several factors (Alvarez *et al.*, 2009) such as level of exposure, duration of

exposure, age, individual response, weight, degree of physical activity and nutrient intake (Fawell *et al.*, 2006). Of course time and amount of urine collected might also determine. In this case morning urine was collected. In our assessment it is observed that urinary fluoride in Fentale and Adamitulu are very high while that of Alaba is almost half of the rest. Fluoride content of the body is not under physiological control (EFSA, 2005) because of the aforementioned factors. Absorbed fluoride is partly retained in bone and partly excreted, predominantly via the kidney. In infants retention in bone can be as high as 90% of the absorbed amount, whereas in adults retention is 50% or less (EFSA, 2006).

The dietary intake of fluoride per body weight per day, assuming 100% bioavailability of ingested fluoride, ranges from 0.25-0.44 (Table 14); the lowest being in Adamitulu. The dietary intake is above tolerable daily intake (TDI) and upper tolerable level (UL) even at 60% bioavailability of ingested fluoride. The Hazard Quotient (HQ) at 60% and 100% bioavailability is above 1 in all the three dietary areas. Based on the Hazard Quotient, the guideline value in respective woredas is also calculated. The guideline value for drinking water calculated is all below that of WHO which is 1.5mg/L. On the other hand, the concentration of fluoride in drinking water is about 5mg/L, i.e, more than 4mg above WHO guideline as well as calculated ones. The result explains that the community is under severe risk of fluorosis. Concentrations above WHO guideline value carry an increasing risk of dental fluorosis, and much higher concentrations lead to skeletal fluorosis (Murray, 1986).

5.9.3 Fluid intake in 24 hrs

Table 15: Fluid intake of children and mother

Study areas	Weight, mean (kg)		Coffee, mean, mL		Water, mean, mL		mean fluid [a] intake, liter		Temperature, mean °C
	C*	M**	C	M	C	M	C	M	
Fentale	26.6	51.4	551	974	938	1139	1.49	2.11	26-38
Alaba	27.4	52.2	233	331.3	736	1004	0.97	1.33	24-29
Adamitulu	32.0	52.6	151	530.1	530	618	0.68	1.15	22-28

C* = Child; M** = mother; [a] the fluid calculated is water and beverage

The total fluid intake by children observed in the study (Table 15) was below the recommendation of both WHO (2011) and academy of science (Whitney and Rolfes, 2005). This might be due to the season of sample collection. The temperature in Rift valley decreases during June - September due to rain fall (Bekele and Amsalu, 2012; Beyene and Gudina, 2009). Normally the fluid intake in hot areas exceeds RDA due to high annual mean temperature. The fluid intake in Fentale is higher than Adamitulu and Alaba. The mean fluid intake correlates with the altitudes and mean annual temperature of the woredas. According to Murray (1986) high fluid intake in hot tropical areas increases the exposure to fluoride and consequently to fluorosis risk. Studies show the development of dental fluorosis (Brouwer *et al.*, 1988) at F^- concentration as low as 0.8 mg/L because of high fluid consumption in hot climatic areas (Fawell *et al.*, 2006; Galagan & Vermillion, 1957). Table 16 shows the guideline value of fluoride in drinking water at different maximum daily temperature.

In the current assessment severe and moderate dental fluorosis was found in Alaba and Adamitulu, the highest being in the later. According to WHO recommendation, based on the temperature range it is clearly observed that fluoride in drinking water should be below 1mg/L.

Table 16: Mean maximum daily temperature and recommended fluoride limit in water

Mean maximum daily temperature (°C)	Maximum fluoride limit (mg/L)
10.0 – 12.0	1.7
12.1 – 14.6	1.5
14.7 – 17.6	1.3
17.7 – 21.4	1.2
21.5 – 26.2	1.0
26.3 – 32.6	0.8

Although drinking water fluoride level was similar and annual mean temperature was higher in Fentale, dental fluorosis is lower than the two water sources. The lower prevalence of fluorosis in Fentale than Adamitulu despite the higher mean annual temperature in the former might be explained in terms of use of rain water and better use of dairy products among Benti communities (Fentale).

According to EFSA (2008a), it is suggested that in setting national standards or local guidelines for fluoride or in evaluating the possible health consequences of exposure to fluoride, it is essential to consider the intake of water by the population of interest and the intake of fluoride from other sources (e.g., food). Where the intakes are likely to approach, or be greater than, 6 mg/day, it would be appropriate to consider setting a standard or local guideline at a concentration lower than 1.5 mg/liter (EFSA, 2008a).

5.9.4 Dental fluorosis rate among study groups

Table 17: Prevalence (%) of dental fluorosis among school age children and mothers

Fluorosis Category	Mother			Children		
	Fentale (N=20)	Alaba (N=100)	Adamitulu (N=96)	Fentale (N=20)	Alaba (N=100)	Adamitulu (N=96)
Normal*	55.0 ^{a}	20.0 ^{b}	39.6 ^{c}	30.0^{d}	7.0^{e}	6.3^{e}
Very Mild	45.0 ^{a}	46.0^{a}	36.5^{a}	45.0^{d}	33.0 ^{e}	12.5 ^{f}
Mild	0.0 ^{a}	24.0^{b}	12.5^{c}	25.0 ^{d}	40.0^{e}	31.3 ^{f}
Moderate	0.0 ^{a}	10.0^{b}	8.3 ^{b}	0.0^{d}	17.0^{e}	39.6 ^{f}
Severe	0.0 ^{a}	0.0 ^{a}	3.1^{b}	0.0 ^{d}	3.0^{e}	10.4^{f}

Cells with different superscripts are significantly different for each group; *Questionable on dean index regarded as normal

As expected, due to dairy, severe and moderate dental fluorosis is not detected in children from Fentale (Table 17). This might be explained because of the differences in milk consumption; high in Fentale and low in Alaba and Adamitulu. The differences in prevalence

of dental fluorosis in Alaba and Adamitulu might be explained with differences in type of cereals used as staple and the use of rain water. Alaba communities are much dependent on finger millet and corn while Adamitulu ones depend on corn and wheat. Moreover, rain water harvesting is common in Alaba and none in Adamitulu.

5.9.5 Skeletal and non-skeletal symptoms in children and their mothers of ERV

Table 18a: Skeletal and non-skeletal symptoms of biological mothers

Skeletal and non-skeletal symptoms in mothers	Fentale (N = 20)	Alaba (N = 96)	Adamitulu (N=100)
% Can't Bend Body& touch Floor/ toe	5.0[a]	7.0[a]	0.0[b]
% Can't Touch Chest with Chin	15.0[a]	17.0[a]	10.6[b]
% Can't Stretch and fold arms to touch back of head	0.0[a]	13.0[b]	0.0[a]
% feel Lower back pain	80.0[a]	31.0[b]	63.5[c]
% feel Tingling sensation	10.0[a]	16.0[b]	28.1[c]
% feel Neck pain (movement)	55. 0[a]	20.0[b]	21.9[c]
% feel Muscle Weakness	15.0[a]	3.0[b]	3.1[b]
% feel Loss of Appetite	10.0[a]	2.0[b]	3.1[b]
% Nausea	0.0[a]	1.0[a]	4.2[b]
% feel Abdominal Pain	0.0[a]	1.0[a]	1.0[a]
% feel Bloated	0.0[a]	1.0[a]	6.2[b]
% feel Polydipsia	0.0[a]	1.0[a]	8.3[b]
% feel Polyuria	0.0 [a]	2.0[a]	2.1[a]
% feel Constipation	0. 0[a]	21.0[b]	24.0[b]

Excessive intake of fluoride during enamel maturation before tooth eruption from birth to eight years of age (when enamel formation is complete) can lead to reduced mineral content of enamel and to dental fluorosis of deciduous but predominantly of permanent teeth. The incidence and severity of dental fluorosis is dose-dependent. Mild dental fluorosis is not readily apparent and is associated with increased resistance to caries. EFSA (2006) considered moderate dental fluorosis, which is characterized by staining and minute pitting of teeth, to be an adverse effect. On the basis that the prevalence of moderate dental fluorosis of permanent teeth is less than 5% in populations ingesting 0.08-0.12 mg fluoride/kg body weight/day, the Panel considered that the upper level (UL) for fluoride is 0.12 mg fluoride/kgbw/day in

children aged 9-14 years (EFSA, 2006).

Table 18b: Skeletal and non-skeletal symptoms of school age children

Skeletal and non-skeletal symptoms in children	Fentale (N = 20)	Alaba (N = 96)	Adamitulu (N=100)
% can`t bend body& touch floor/toe	0.0[a]	12.0[b]	0.0[a]
% can`t touch chest with chin	5.0[a]	12.0[b]	1.0[c]
% can`t stretch and fold arms to touch back of head	0.0[a]	14.0[b]	0.0[a]
% feel Lower back pain	10.0[a]	9.0[a]	11.5[a]
% feel Leg Pain, joints	10.0[a]	4.0[b]	4.2[b]
% feel Arm Pain, joints	5.0[a]	4.0[a]	5.2[a]
% feel Tingling sensation	5.0[a]	5.0[a]	4.2[a]
% feel Neck pain, movement	5.0[a]	5.0[a]	4.2[a]
% feel Muscle Weakness	5.0[a]	2.0[a]	0.0[a]
% feel Loss Appetite	0.0[a]	0.0[a]	2.1[a]
% feel Feelings of Nausea	0.0[a]	0.0[a]	1.0[a]
% feel Abdominal Pain	0.0[a]	1.0[a]	4.2[a]
% feel Bloated	0.0[a]	1.0[a]	2.1[a]
% feel Polydipsia, excessive thirst	5.0[a]	0.0[b]	2.1[b]
% feel Polyuria, large volume of dilute urine	0.0[a]	0.0[a]	0.0[a]
% feel Constipation	0.0[a]	3.0[a]	10.4[b]

In this study (Table 18), it is attempted to figure out if there are clinical symptoms of skeletal and non-skeletal fluorosis existed. The result of the investigation showed that some stiffness of joints existed in all the study areas. Constipation is also observed in 10% of children in Adamitulu. The other commonly observed symptoms include tingling sensation in the extremities, stiffness in the neck movement and muscle weakness. As developed by Susheela

and colleagues (2002), clinical symptoms in arms, lower back bone and neck is lower in children. These symptoms prevail more in adults than children.

5.9.6 Urinary fluoride

Table 19: Urinary fluoride level at various cut-offs in children and their mothers

Urinary fluoride	Mothers			children		
Cut-off points	Fentale	Alaba	Adamitulu	Fentale	Alaba	Adamitulu
≤2.5	25.0 [a]	30.0 [b]	10.0 [c]	10.0 [a]	40.0 [b]	5.0 [c]
2.6-5	15.0 [a]	35.0[b]	10.0[c]	30.0 [a]	45.0[b]	15.0 [c]
>5	60.0[a]	35.0 [b]	80.0 [c]	60.0 [a]	15.0 [b]	80.0 [c]

***Cut off points is based on the assumption of 5mg /day upper intake and 50% urinary fluoride excretion (EFSA, 2006); C* = child; M** = mother

Fluoride accretion in bone increases bone density but long term intake reduces bone strength and increases risk of fracture (Turner *et al.*, 1995; Chachra *et al.*, 1999) and skeletal fluorosis. The Panel applied an uncertainty factor of 5 to derive an UL of 0.12 mg/kg body weight/day. This is equivalent to an UL of 5 mg/day in children aged 9-14 years (EFSA, 2006).

The urinary fluoride is one of the biomarker for ingested fluoride, although the balance depends on several factors. Assuming 50% excretion of ingested fluoride through urine, it is observed (Table 19) that about 60% in Fentale, 80% in Adamitulu and 15% in Alaba have more than 5mg/L urinary fluoride. Assuming 50% excretion of absorbed fluoride, the people are ingesting more than 10mg fluoride per day. The result was consistent with dietary fluoride ingested the day before collection of the urine.

5.10 Nutrient intake

The nutrient intake of children and their mothers in the study areas were determined. Researchers (Susheela and Bhatnagar 2002) claim that nutritional deficiency is one of the risk factor for fluorosis. Especially the lack of sufficient calcium and anti-oxidants increases the risk. The mineral intakes of the subjects are presented in table 20. Calcium in the diet of the subjects was low. Iron was the most abundant nutrient in the diet of the studied subjects. About 30% of children in Fentale, 100% in Alaba and 45% in Adamitulu had an estimated iron intake above tolerable level (UL). The most limiting mineral in the diet of children in each area was zinc. The finding is consistent with Ethiopian Food consumption survey conducted in 2010 (EHNRI, 2013).

Calcium consumption is below RDA in Fentale (100%) and in Adamitulu (95%). In Alaba, only about 30% of children and 20% of mothers show calcium consumption below RDA, in fact about 15% of children and 35% of mothers had got calcium above upper tolerable level in Alaba. Consumption of phosphorus is very low in Fentale and fair in Alaba and Adamitulu. Consumption of the nutrients by children is consistent with their mothers in each woreda.

Table 20: Nutrient intake of children and their mothers per day by cut off points

Parameters	Cut off points	Children			Mothers		
		Fentale	Alaba	Adamitulu	Fentale	Alaba	Adamitulu
Iron	Below RDA	0^a	0^a	0^a	5^a	0^b	10^c
	Sufficient	70^a	0^b	55^c	80^a	0^b	50^c
	Above UL	30^a	100^b	45^c	15^a	100^b	40^c
Zinc	Below RDA	65^a	10^b	25^c	65^a	0^b	5^c
	Sufficient	35^a	60^b	75^c	35^a	90^b	95^b
	Above UL	0^a	30^b	0^a	0^a	10^b	0^a
Calcium	Below RDA	100^a	30^b	95^c	100^a	20^b	90^c
	Sufficient	0^a	55^b	5^c	0^a	45^b	10^c
	Above UL	0^a	15^b	0^a	0^a	35^b	0^a
Phosphorus	below 1250	90^a	15^b	35^c	35^a	10^b	0^c
	1250 – 4000	10^a	85^b	65^c	65^a	85^b	100^c
	>4000	0^a	0^a	0^a	0^a	5^b	0^a

About 40 % of Fentale children (Table 20) and 15% of Adamitulu had protein and energy consumption below RDA for their age. Children in all the study areas had optimal daily calorie. Daily phosphorus consumption below RDA (1250mg) is 90% among children in Fentale. Low consumption of phosphorus by children is only 15% in Alaba and 35% in Adamitulu.

Table 21: % children and mother got adequate calorie intake

	% Mother				%Children			
Nutrient	**Cut-off point**	**Fentale (20)**	**Alaba (20)**	**Adami T (20)**	**Cut-off points**	**Fentale (20)**	**Alaba (20)**	**Adami T (20)**
Fiber,	< 25	95.0[a]	15.0[b]	80.0[c]	<26	100.0[a]	25.0[b]	95.0[c]
	≥ 25	5.0[a]	85.0[b]	20.0[c]	≥ 26	0.0[a]	75.0[b]	5.0[a]
Protein	< 46	70.0[a]	0.0[b]	35.0[c]	< 34	40.0[a]	0.0[b]	15.0[c]
	=/> 46	30.0[a]	100.0[b]	65.0[c]	≥ 34	60.0[a]	100.0[b]	85.0[c]
CHO = 0.000	< 130	60.0[a]	0.0[b]	0.0[b]	< 130	50.0[a]	0.0[b]	10.0[c]
	≥130	40.0[a]	100.0[b]	100.0[b]	≥ 130	50.0[a]	100.0[b]	90.0[c]
Energy	< 2200	100.0[a]	65.0[b]	95.0[a]	< 2071	40.0[a]	0.0[b]	15.0[c]
	2200-2402.9	0.0[a]	10.0[b]	0.0[a]	2071-2279	60.0[a]	100.0[b]	85.0[c]
	≥ 2403	0.0[a]	25.0[b]	5.0[a]	> 2279	0.0[a]	0.0[a]	0.0[a]

In the current assessment (Table 21) the fiber level in Fentale and Adamitulu is below recommended daily allowance of 26g for the age group of the children. Generally, among the studied woredas children in Fentale have low dietary intake of nutrients and that of Alaba have better nutritional provision. Mothers have better calorie consumption than the children.

Consumption of Food Groups (HDDS)

Dietary Diversity assessment was conducted from 24 hr recall and duplicate plates. Both data are consistent. Consumption of milk and milk products (Table 22) are common in Fentale (100%) and very low in Alaba (41%) and Adamitulu (22.3%). The consumption of beverage (coffee) in all study sites is high above 80%. The consumption of antioxidant rich foods such as vegetables and fruits are very low. Kale is common in Alaba and Adamitulu, and none in Fentale. Vitamin C and A are mentioned elsewhere to help in minimizing fluorosis risk

(Susheela and Bhatnagar, 2002). Kale which is rich in anti-oxidants might help in the mitigation of fluorosis.

Table 22: HDDS from 24 hr recall

% food groups consumed	Fentale	Alaba	Adamitulu
Cereals	100.0	99.0	98.9
Vitamin A Rich Roots	0.0	1.0	1.1
White Roots and Tubers	0.0	2.0	16.0
Green Leafy Vegetables	0.0	86.0	91.5
Vitamin A Rich Fruits	0.0	0.0	1.1
Other Vegetables	0.0	10.0	93.6
*Other Fruits,	0.0	0.0	0.0
*Iron Rich Organ meat, Meat and Fish	0.0	0.0	0.0
Egg	0.0	3.0	0.0
Legumes	45.0	43.0	22.3
Milk and milk products	100.0	41.0	22.3
Oil and Fats	100.0	23.0	98.9
Red Palm and Products	5.0	0.0	0.0
Sweet Foods	45.0	0.0	0.0
Spice, beverage and Condiments	80.0	93.0	89.4
Ate out of Home	5.0	0.0	1.1

* Not consumed at all in all the studied areas

Over 43 % of HHs in Alaba, less than 10 % in Adamitulu and none in Fentale had dietary diversity below 4 (poor) based on FAO cut-off points (Table 23). Medium dietary diversity was observed in Fentale and Adamitulu. High dietary diversity was practiced by less than 10 % of the study subjects in Alaba and Adamitulu. Since dairy product was one of the diets in Fentale, all the subjects lie in the medium range.

Table 23: HDDS and Child Dietary Diversity Score

	Category	Fentale	Alaba	Adamitulu
HDDS	Low (≤ 3)	0.0[a]	43.0[b]	7.4[c]
	Medium (4-5)	100.0[a]	51.0[b]	81.9[c]
	High (> 5)	0.0[a]	6.0[b]	10.6[c]
Child DDS	Low (1-3)	100.0[a]	87.0[b]	55.4[c]
	Medium (4-5)	0.0[a]	13.0[b]	44.6[c]
	High (>5)	0.0[a]	0.0[a]	0.0[a]

Cells with different superscripts are significantly different across row

Although the DDS (Table 23) of Fentale children is poor, since dairy product is one of the foods mainly consumed in the area, it can't underestimated in terms of mitigating ingested fluoride. Dairy products were mentioned elsewhere to reduce risk of fluorosis in addition to providing most of the nutrients needed in human diet. Children DDS is poor (<4) in Fentale (100%) and Alaba (87%). None of the children in the study areas had high dietary diversity (>5).

Table 24: Vitamin A & Iron Rich foods consumption by households

% Consumed	% Fentale	% Alaba	% Adamitulu
Vitamin A Plant Based Food	0.0[a]	88.0[b]	93.6[c]
Vitamin A Animal Based Food	100.0[a]	19.0[b]	23.4[c]
Haem Iron Food	0.0[a]	0.0[a]	0.0[a]

Haem iron consumption (Table 24) is entirely nil among the study subjects. The consumption of plant based vitamin A rich foods in Fentale is nil while it is above 85% in Alaba and Adamitulu. Consumption of dairy products in Fentale is 100%. This might helped the inhabitants to mitigate ingested fluoride from water.

Table 25: Water and Sanitation

Variables		Fentale (N=20)	Alaba (N=100)	Adamitulu (N=96)
Source of drinking water		Motorized borehole	Wind mill borehole	Wind mill borehole
% HHs who fetch water within an hour		25.0 [a]	0.0 [b]	99.0 [c]
% HHs using Water defluoridation		0.0 [a]	1.0 [a]	0.0 [a]
% HHs who use Water Disinfectant		55.0 [a]	6.0 [b]	0.0 [c]
% HHs who harvest rain Water		0.0 [a]	25.0 [b]	0.0 [a]
% months of rain water use	Not at all	0.0 [a]	75.0 [b]	100.0 [c]
	1-3 months	0.0 [a]	24.0 [b]	0.0 [a]
	4-6 months	100.0 [a]	1.0 [b]	0.0 [b]
water storage apparatus	"Insira" / Clay	0.0 [a]	9.0 [b]	0.0 [a]
	Jerican / Plastic	100.0 [a]	91.0 [b]	100.0 [a]

Cells with different superscripts are significantly different across the row

All the three study areas got water from either wind mill or motor generated bore hole (Table 25). All of the waters are slightly hot. Rain water harvesting is rare. In Fentale, people don't harvest rain water but they use the one accumulated by itself from heavy rain fall. The use of harvested rain water in Fentale covers over 4 months. In Alaba, 25% of the households harvested rain water and use for about 3 months. Adamitulu communities have no practice of harvesting and using rain water. The government of Ethiopia is promoting rain water harvesting as one strategy for food security (USDA, 2010). With regard to reduction of fluorosis the practice is almost nil. Those who harvest rain water, they do so to reduce time to go and fetch water and not for reduction of fluorosis or for food security purpose. The practice of using disinfectant was about 55% in Fentale (Benti) and none in the rest. The study subjects store water in a plastic container (Jerican). Among the study subjects, 99% of Adamitulu and 25% of Fentale fetch water within an hour time while Alaba HHs travel more than an hour.

5.11 Standard reference materials and spiked specimens for Method validation

The validation of method was conducted using spiked samples and analysis of reference materials. The recoveries of spiked samples show that the method is good (% error < 10%). The analysis from reference material also shows within the range of company standard deviation (Table 26).

Table 26: Standard reference materials and spiked samples for method validation

Sample type	N	Non spiked, µgF	spiked, µgF	Reading	% recovery
Feces, rat	3	3.4	5	8.6	104.4
Urine, rat	3	4.6	5	9.5	98.6
Brewed tea (anbesa tea)	3	4.4	5	9.6	104.5
Standard Reference		**At company**		**At our lab**	
material		Mean	Stdev.	Mean	Stdev.
SRM 1 (Plant tissue)	9	277	27	282	17
SRM 2 (Fish tissue)	9	25	not reported	29	4

5.12 Results from animal trial

The mean daily consumption of water by the rat was 18mL and the mean daily consumption of food was 15gram. From figure 13, it is clearly observed that the weights of all the rats increased. The percent increment of moringa or calcium supplemented ones were more than the control ones. When we compare the weights among the control fluoride supplemented / contaminated show more weight increment than those not supplemented (FF).

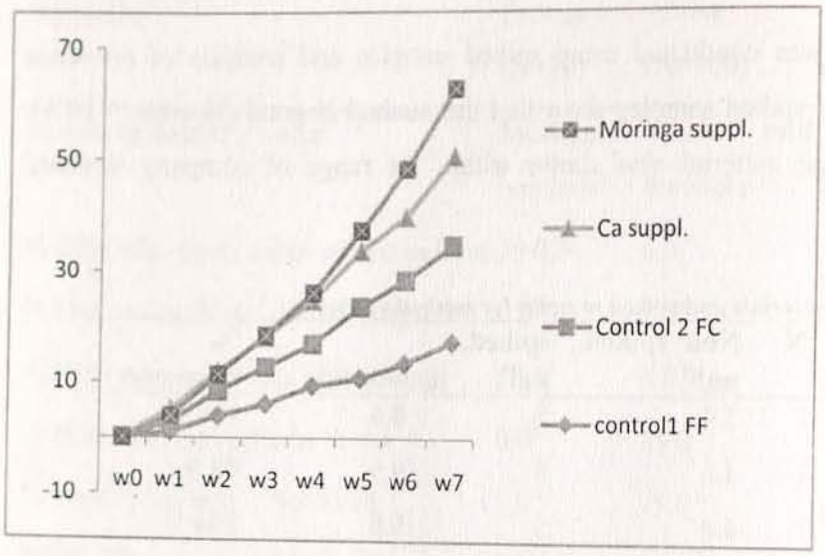

Figure 13: Percent increment of weights of rats

5.12.1 Fluoride in feces of rat supplemented with calcium or Moringa for 6 weeks

Concentration of fluoride in feces of rats supplemented with calcium or moringa was higher than that of control. The controls (FF and FC) differed from the treatment group by supplements. The fluoride in feces of nutrient supplemented group was twice as the control group (FC). The fluoride concentration of the control is twice as the non-contaminated rats (Table 26).

Table 26: ANOVA analysis: fecal fluoride (mg/kg) of rats over 6 weeks

Supplement	N	Mean	SD.
FF: Normal diet, Non-Fluoridated water	6	3.6^{a*}	1.0
FC: Normal diet, Fluoridated water	6	6.4^{a}	0.8
CA: Calcium supplemented diet, Fluoridated water	6	14.7^{b}	4.8
M: Moringa supplemented diet, Fluoridated water	6	13.1^{b}	4.0

*Different superscripts are significantly different

ANOVA shows that feces fluoride has significant difference (p=0.002) between calcium or moringa supplemented and non-supplemented. On the other hand fluoride content of feces of

rats contaminated and non-contaminated has no significant difference (p=0.150). The comparison of means indicate tha in absence of nutrient (calcium) in the diet the excretion of fluoride is mainly through urine. the excretion is shared among feces and urine when calcium s supplemented.

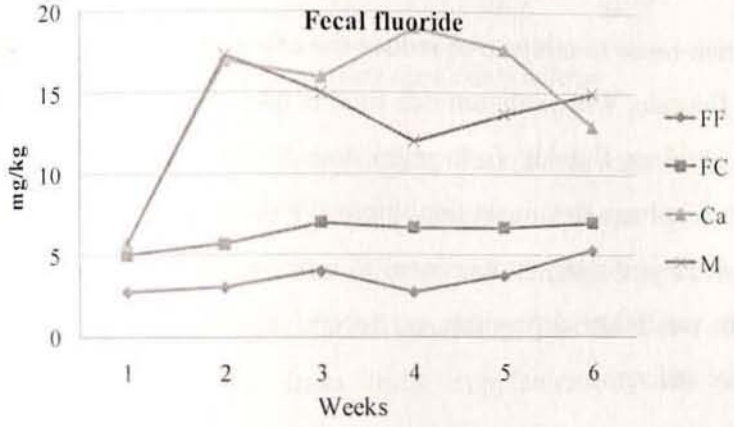

Figure 14: Fluoride in feces of rats supplemented with calcium or moringa

When fluoride excretion is compared among the treatments, mean fluoride excreted by supplementation of calcium or moringa was not significantly different (p=0.395). But the control was significantly different from moringa supplemented (p=0.002) as well as calcium supplemented (p=0.000). The excretion of fluoride by rats consumed fluoridated water (fluoride contaminated/control) was not significantly different (p=0.150) from non-fluoridated (FF). This shows that the excretion of fluoride through feces depended on amount of nutrients taken, especially calcium.

Fluoride combining elements that form insoluble compound if consumed in diet/beverages are used to excrete fluoride via feces. The graph clearly shows the potential benefit of nutrients in mitigating ingested fluoride (Figure 14). Level of fluoride in feces of rats supplemented with moringa or calcium is almost double compared to the control groups. The Rift valley communities which are living under fluoride burden due to contaminated water with fluoride should need to diversify their diet with calcium and anti-oxidant rich foods. If milk is not available or scarce, moringa can substitute calcium rich foods if customized in the diet of

endemic communities and could be used for the mitigation of ingested fluoride. Moreover since moringa and vegetables like kale are rich in anti-oxidants (Vit C, A,) using these foods may also reduce the toxicity that might be caused due to fluoride.

Fluoride in urine of rats supplemented with calcium or moringa

The principle of using calcium rich foods to mitigate or reduce the effect of ingested fluoride lies on the solubility of calcium fluoride. When calcium rich food is taken along with fluoride contaminated water, calcium combines fluoride (scavenge) and forms insoluble calcium fluoride compound. By doing so it reduces the absorption/ bioavailability of fluoride (Figure 15). The amount of fluoride in urine is directly proportional to amount of fluoride absorbed although the balance has great variability depending on several factors like amount of exposure, duration, nutrient intake and age.

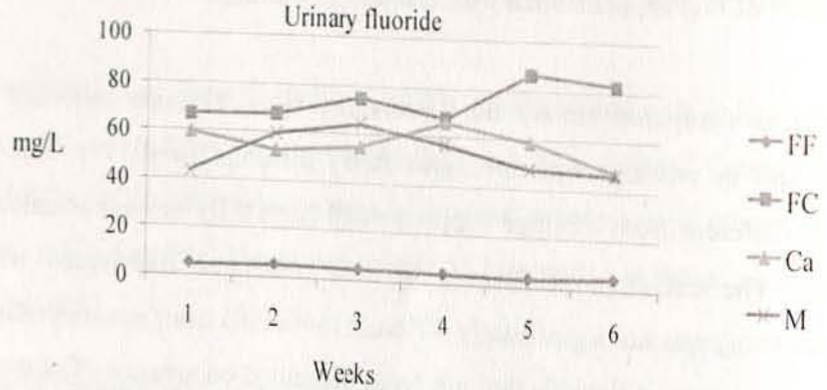

Figure 15: Urinary fluoride of rats supplemented with Calcium or Moringa over 6 weeks

During early exposure rate of retention in bone and teeth is high (about 90%) and the rate decreases as much fluoride is absorbed or adsorbed. The amount of fluoride in feces is directly proportional to amount of calcium intake. It is mean that consumption of calcium with fluoridated water decreases urinary fluoride and increases feces fluoride. This is what exactly observed in this animal trial.

Table 27: ANOVA: Urinary fluoride of rats

Group	N	Mean	Stdev.	Min	Max
FF	3	4.6 [a*]	0.6	3.8	5.6
FC	3	76.0 [b]	8.5	67.8	86.6
CA	3	55.9 [c]	6.9	47.4	66.0
M	3	55.2 [c]	7.6	46.6	64.5

* Values with different superscripts are significantly different

The urinary fluoride of rats drinking fluoridated water is far above those of non-fluoridated water (p=0.000, Table 27).Urinary fluoride decreases upon supplementation of nutrients. In this study it is observed that calcium and moringa supplemented rats have got lower urinary fluoride than control (p=0.000).The urinary fluoride of rats supplemented with moringa is not significantly different from those supplemented with calcium citrate (p=0.410). The investigation show that the possibility of using moringa as a substitute for calcium supplement. The blending of moringa with flour increases its calcium level. In fact, it also raises the anti oxidant level, as moringa leaf is rich in Vit A, C, Iron and protein.

The increase of fecal fluoride content and decrease of urinary fluoride on supplementation of calcium and or calcium rich foods strongly suggests that fluoride combines with calcium to form insoluble compound. The percent increase of concentration of fluoride in feces and percent decrease in urine confirms that supplementation of calcium creates fluoride inbalance although the *stoichiometry* is not exactly equivalent because of the insolubility of some amount of calcium fluoride in water and other factors.

5.13 Ingested fluoride mitigation through nutrient supplementation: human trial

The daily urinary fluoride of women show that supplementation of milk, calcium tablet or moringa dried leaf reduces urinary fluoride when compared with control (Figure 16).

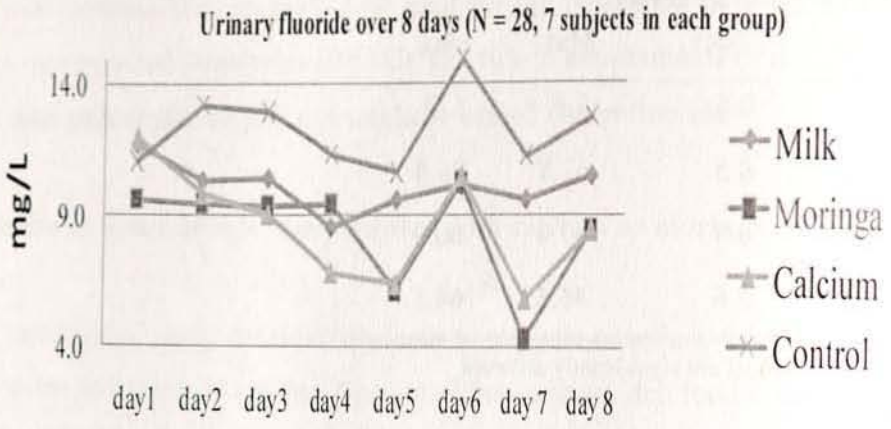

Figure 16: Urinary fluoride of women supplemented with Ca-rich foods over 8 days

The level of fluoride concentration in urine of control group is higher than those treated with milk, moringa or calcium (Table 28). Figure 16 clearly shows the potential use of nutrient supplementation in mitigating ingested fluoride and consequently decreases the degree of fluorosis. The milk supplemented group show relatively higher level of urinary fluoride than moringa or calcium tablet supplemented ones. The difference in urinary fluoride level between bread made of moringa dry leaf powder or bread with calcium tablet blended wheat powder) and milk might be attributed to the state of the foods and the time difference in the absorption of both fluoride (in water) and calcium (in bread or milk).

Table 28: Urinary Fluoride

Treatment groups	N	Mean	SD	Min	Max
Moringa	8	8.3^{a*}	2.1	4.3	10.2
Calcium	8	8.6^{a}	2.1	5.8	11.9
Milk	8	10.1^{b}	0.9	8.5	11.7
Control	8	12.2^{c}	1.5	10.6	14.9

* Values with different superscripts are significantly different

CHAPTER SIX: CONCLUSIONS AND RECOMENDATIONS

6.1 Conclusion

- Fluoride both from water as well as food is high. The daily fluoride intake from water alone exceed the guideline value given by WHO(2008). The fluoride content of food ingredients and cereals such as teff, some tea brands and chili powder had the highest fluoride content. In all study areas the daily fluoride intake exceeds the upper tolerable level of 0.1mg/bw/day recommended by EFSA (2008).
- The altitude and mean annual temperature in the area will induce water consumption to adjust to the hot environment. The high water consumption will expose the community to higher fluorosis risk. As a result severe and moderate dental fluorosis and some forms of skeletal fluorosis such as stiffness of joints, tingling sensation in the extremities, stiffness in the neck movement and muscle weakness are common in all the study areas. On the other hand dietary practice of calcium rich foods, anti-oxidant rich fruits and vegetables and dietary diversity of the community is poor.
- Among the study sites low severe dental fluorosis rate is observed in those who harvest rain water and those who have dietary practice of calcium rich foods; dairy products.
- The study indicates that the health consequences of fluoride contaminated water is fairly understood but still there is a knowledge gap and wrong perception concerning fluoride and its health consequences among the community including health extension workers. Less emphasis was given to fluoride contamination in the presence of other infectious and non-infectious diseases.
- Both in rats and reproductive age women, moringa and calcium supplement reduced urinary fluoride. In the rat study, increased fecal fluoride levels were found. These experimental trials indicate that fluoride bioavailability can be reduced by complementing diet with calcium rich foods.

6.2 Recommendation

- In areas where provision of fluoride free water is impossible the communities need to use defluoridation techniques and rain water harvesting. Moreover foods that have high fluoride content must be avoided and those foods which have low fluoride level should be promoted. Therefore use of dairy products, finger millet and vegetables that are rich in calcium need to be promoted in the areas. Teff is also a good source of calcium but due to high soil content, difficult to purify soil from grain of teff, it is also fluoride contaminated. Moreover, such plants like moringa which is suitable to cultivate in rift area and rich in calcium and antioxidants may also help in mitigating ingested fluoride as well as in reducing risk of fluorosis.
- The trend of harvesting and using rain water should be encouraged because it will reduce the fluoride intake. Rain water harvesting will also help as a means to make gardening and use anti-oxidant rich vegetables to reduce the toxic effect of fluoride and better use of the nutrients.
- The health consequences of fluoride contamination and mitigatory methods should get sufficient attention by the community, health workers and concerned governmental bodies through appropriate health interventions including information communication. Ministry of health should give attention and coordinate the mitigation actions.
- Fluorosis mitigation and prevention should be part of the work of health extension workers in the Main Ethiopian rift valley. Moreover information communication works among community as well as health promoters including health extension works should be given more emphasis in future mitigation works. Future studies should focus on interventions on defluoridation and mitigatory techniques. Beside defluoridation and rain water harvesting, nutrients intake and dietary habit adjustment might help to safe guard the Rift valley community.
- Since the community have positive attitude in taking an active part in future efforts in providing the community with a safe water, stakeholders and concerned governmental bodies should participate the community, support and mobilize resources in provision of safe and fluoride free water.

6.3 Limitations of the study

- The study had limitation of using small sample size for dietary intake and absence of confirmation of skeletal fluorosis with radiological methods.
- The assessment was conducted at the beginning of summer (June, 2013). During dietary intake assessment there was seasonal scarcity of pasture in Fentale (Benti community) and most of the households were migrated in search of grazing land until the rain falls and the area regenerate. Consequently only 20 households were included in the assessment.
- Since milk was scarce, in the study areas it was excluded from duplicate plate technique and dietary intake assessment of milk was conducted based on food frequency questionnaire. Hence its nutrient contribution was not included in the study.

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APPENDICES

7.1 Consent form and tools used for data collection

የጥናቱ ርዕስ፡- በምግብና ውሃ አማክኝነት በሰውነት ውስጥ የሚገባው ፍሎራይድ መጠን፣የሚያስከትለው ችግርና መፍትሔው

ቅጽ 1 ፡- የቤተሰብ መጠየቅ

የፍቃደኝነት መግለጫ

እኔ---------- አባላለሁ። የመጣሁት ከጤና ጥበቃ ሚ/ር ነው። ሚኒስቴር መ/ቤቱ የህብረተሰቡን ጤና ለማሻሻል እየሰራ ነው። በመሆኑም ጥራቱ ያልተጠበቀ ውሃ በመጠጣት የሚመጣውን የጤና ጉዳት መጠንና ስፋት ለማጥናትና የመፍትሔ ሀሳብ ለመጠቆም ምርምር እያከሄድን ነው። የእርስዎም ቤተሰብ የዚህ ጥናት አባል ሆኖ በመመረጡ ደስ ይለናል። እርስዎ የሚሰጡን መረጃዎች በሙሉ ሚስጢራዊነታቸው የተጠበቀ ነው። ለቃለመጠየቁ ከአስር እስከ አሥራአምስት ዓመት የሆነ ልጅና እናቱ / አሳዳጊ እንፈልጋለን። የጥርስ ምርመራም እናደረጋለን። የጠዋት ሽንትና የሙሉ ቀን የምግብ ናሙናም እንፈልጋለን። ለሚሰጡን የምግብ ናሙና በመስሪያ ቤታችን የተመደበውን ተመጣጣኝ ገንዘብ እንሰጣለን። በዚህ ምርምር ላይ ለመሳተፍ ፈቃደኛ ነዎት?።

(ስምምነትዎን ይግለጹ) (1 = አዎ 0 = አይደለም) _____

መሳተፍ ካልፈለጉ ላለመሳተፍ የተሰጠው ዋና ምክንያት፡ _____

ማሳሰቢያ፡- ፈቃደኛነት ከተረጋገጠ ቃለ-መጠየቁን ማካሄድ ካልሆነ አመስግኖ መሰናበትና ሁኔታውን ለአስተባባሪው ማሳወቅ።

ክፍል1፡- የቤተሰብና የአከባቢ መለያ ቅፅ

1	መለያ ቁጥር	
2	ክልል	
3	ዞን	
4	ወረዳ	
5	ቀበሌ	
6	የቤተሰብ አስተዳደሪ ስም	
7	የተጠያቂ ስም	
8	ቋንቋ	
9	በአከባቢው ለምን ያህል ዓመት ኖሩ	
10	አጠቃላይ የቤተሰብ ብዛት	
11	5 ዓመትና ከዚያ በታች	
12	ከ6-15 ዓመት	
13	ከ15 ዓመት በላይ	
14	የመረጃ ሰብሳቢ ስም፡	ፊርማ

ክፍል2፡- የልጅ አወላለድን በተመለከተ

15	ስንት ጊዜ አርገዚሽ/ው ያውቃሉ?	
16	ስንት ልጆች አሉሽ/ሎት?	
17	የወልድ ችግር አጋጥሞሽ ያውቃል?	
18	ካለ ምን ዓይነት ችግር?	
19	ውርጃ አጋጥሞሽ ያውቃል?	
20	ያለመፀነስ?	
21	በማህፀን ውስጥ መሞት?	
22	የአካል ችግር?	
23	የአካል ችግር በልጆች ላይ ካጋጠመ የችግሩ ዓይነት	

ክፍል 3፡- ሀብት (ከብቶች)

		a)	b)
24	የቤት እንስሳት በቁጥር?	አዎ = 1 የለም= 0	ብዛት?
25	Camel/ግመል		
26	Goat/Sheep /በግ/ፍየል		
27	Cattle /ከብት (ላምና በሬ)		
28	Donkey/Mule /አህያ/በቅሎ/ፈረስ		
29	ዶሮ		

ክፍል4፡- MILK CONSUMPTION / የወተት አመጋገብ

		in local unit	mL
30	By child per day/ ልጅ በቀን ውስጥ		
31	By mother per day/ የእናት በቀን ውስጥ		

ክፍል 5፡- የቤተሰብ ምግብ ዋስትና መጠይቅ

32	ባለፈው አንድ ወር ውስጥ ቤተሰብዎ በቂ ምግብ የለውም ብለው ተጨንቀው ያውቃሉ?	1 = አዎ 0 = አይደለም	
33	ባለፈው አንድ ወር ውስጥ እርሶ ወይም ከቤተሰቦ አንዳቸው በማጣት ምክንያት የሚፈልጉትን ምግብ መብላት ፈልገው ሳይበሉ ቀርተው ያውቃሉ?	1 = አዎ 0 = አይደለም	
34	ባለፈው አንድ ወር ውስጥ እርስዎ ወይንም ከቤተሰቦ አንዳቸው በማጣት ምክንያት የተወሰኑ ምግቦችን ብቻ ደጋግመው እንድበሉ ሆነው ያውቃሉ?	1 = አዎ 0 = አይደለም	
35	ባለፈው አንድ ወር ውስጥ እርስዎ ወይንም ከቤተሰቦ አንዳቸው ሌሎች የምግብ አይነቶችን ለማግኘት ባለመቻላችሁ በፍጹም መብላት የማትፈልጓቸውን ምግቦች እንድትበሉ ተገዳችሁ ታውቃላችሁ?	1 = አዎ 0 = አይደለም	
36	ባለፈው አንድ ወር ውስጥ እርስዎ ወይንም ከቤተሰቦ አንዳቸው በቂ ምግብ ባለመኖሩ ምክንያት በቀን ውስጥ መመገብ አለብኝ ብላችሁ ከምታስቡት ያነሰ እንድትመገቡ ሆናችሁ ታውቃላችሁ?	1 = አዎ 0 = አይደለም	
37	ባለፈው አንድ ወር ውስጥ እርስዎ ወይንም ማንኛውም የቤተሰቡ አባል በቤት ውስጥ በቂ ምግብ ካለመኖሩ የተነሣ ቀኑን ሙሉ ጥቂት ምግብ ብቻ እንድትመገቡ ሆናችሁ ታውቃላችሁ?	1 = አዎ 0 = አይደለም	
38	ባለፈው አንድ ወር ውስጥ በማጣት የተነሣ በቤትዎ ውስጥ ምንም አይነት የሚበላ ምግብ ጠፍቶ ያውቃል?	1 = አዎ 0 = አይደለም	
39	ባለፈው አንድ ወር ውስጥ እርስዎ ወይንም ከቤተሰቦ አንዳቸው በቂ ምግብ ባለመኖሩ ምክንያት እራት ሳይበሉ እንዲተኙ ሆነው ያውቃሉ?	1 = አዎ 0 = አይደለም	
40	ባለፈው አንድ ወር ውስጥ እርስዎ ወይንም ከቤተሰቦ አንዳቸው ቀኑን ሙሉ(ማታ እና ቀን) ምንም አይነት ምግብ ሳይበሉ ቀርተው ያውቃሉ?	1 = አዎ 0 = አይደለም	

ክፍል 6፡- ውሃና ንፅህና መጠየቅ / WATER TREATMENT PRACTICE

41	የቤተሰቡ ዋነኛ የመጠጥ ውሃ ምንጭ ምንድን ነው?	1. ጉድጉድ ውሃ 2. በእጅ /ንፋስ የሚዘወር የጉድጉድ ውሃ 3. የተጠራቀመ የዝናብ ውሃ 4. ሌላ ----------
42	ውሃ ቀድቶ ለመመለስ ምን ያክል ጊዜ ይወስዳል?	ከአንድ ሰኣት በታች.....1 ከአንድ ሰኣት በላይ ..... 2 አይታወቅም.....3
43	ቤተሰቡ የመጠጥ ውሃን ንፅህና ለመጠበቅ የሚደርገው ጥረት አለ? (አቃ ማጠብን አይመለከትም)?	አዎ ሁልጊዜ.....1 አዎ አንዳንድ ጊዜ.....2 የለም.....3
44	ለጥያቄ ቁጥር 3 መልሱ አዎ ከሆነ የውሃውን ንፅህና ለመጠበቅ የሚጠቀሙት ዘዴ ምንድን ነው? *(ከአንድ በላይ መልስ ሊሰጥ ይችላል)*	ውሃን ማፍላት.....1 ውሃ አጋር/ክሎሪን መጨመር.....2 በጨርቅ ማጥለል.....3 ቀሻሻው እንዲዘቅጥ በማድረግ.....4 ሌላ/(ይገለ).....5
45	For cooking / ምግብ ለማዘጋጀት የሚጠቀሙት ከመጠጥ ውሃው ይለያል?	
46	For bathing/ ለንፅህና የሚጠቀሙት ውሃ ከመጠጥ ውሃው ይለያል?	
47	Water storage; clay, plastic other / ቤት ውስጥ ውሃን በምን ያኖራሉ?	
48	Trends of treating for fluoride removal or fluorosis mitigation ፍሎራይድን ከውሃ ውስጥ ለመቀነስ/ ለማጥፋት የሚያደርጉት ጥረት አለ?	
49	Trends of harvesting rain water የዝናብ ውሃን ያጠራቅማሉ?	
50	Months of using harvested rain water የዝናብ ውሃን ለምን ያህል ወራት ይጠቀማሉ?	

ክፍል 7:- ANTHROPOMETRIC DATA /የክብደትና ቁመት ክንድ ዙሪያ ቅፅ(ኤ.ኤን)

1	2		3	4	5	6	7
ተ.ቁ	ስም	ፆታ	መለያ ቁጥር	ዕድሜ	ክብደት በ ኪ.ግ	ቁመት (በሴ.ሜ/ር)	ሙዋክ (በሴ.ሜ/ር)
1-እናት		ሴ					
2-ልጅ							

ክፍል 8፡- የቤተሰብ የተለያዩ ምግብ የአመጋገብ ግምገማ (IDDS)

ይህንን ጥያቄ የምትጠየቀው እማወራ ወይም የቤተሰቡን ምግብ ዝግጅት የሚታውቅ መሆን አለባት፡ለትውስታ እንዲመች አሁን ካለችበት ሰዓት ወደ ኃላ መጠየቅ ይቀላል። የ24 ሰዓቱን ስትጠይቅ በአያንዳንዱ የምግብ ሰዓት የተመገቡትን በጥያቄው በሰተግራ አስፍርና እንደየምግቡ ምድብ ለያቸው። ለዚህ ሪድፍ ለተመገቡት ምግብ "1"ን ላልተመገቡት ምግብ ደግሞ "0"ን በተሰጠው ቦታ ላይ ፃፍ።

	1	2-ልጅ	3-እናት	ባለፉት 24 ሰዓት የተበሉ ምግቦች
ተ.ቁ	**የምግብ ዓይነት/መግለጫ**	(0 /1)	(0/1)	
1	**የአገዳ እህሎች፡-**ከጤፍ ፤ሩዝ ፤ገብስ፤ ስንዴ፤ በቆሎ፤ ማሽላ፤ ዳጉሳ፤አጃ፤ የተዘጋጀ (እነጀራ፤ቂጣ, ገንፎ፤ ዳቦ፤ አንባሻ፤ ፓስታ፤ብስኩት, ኩኪስ?			**ለእናት**
2	**በቫይታሚን ኤ የበለፀጉ አትክልቶችና ሥራሥሮች፡-**ዱባ፤ካሮት፤ በውስጡ ቢጫ/ብርቱካናማ ቀለም ያለው ስኳር ድንች፤			
3	**ነጣ ያሉ ሥራሥሮች፡-** ነጭ ድንች፤ ጎደሬ፤ነጭ ካሳቫ፤ቡላ፤ቆጮ፤ ሌሎች ስራስሮች?			
4	**ጠቆር ያለ አረንጉዴ ቅጠል ያላቸው አትክልቶች፡-**ጠቆር ያለ አረንጉዴ ቅጠል ያላቸው አትክልቶች (ጥቁር ጎመን/የህበሻ ጎመን/ የሽፈራው ቅጠል)			
5	**በቫይታሚን ኤ የበለፀጉ ፍራፍሬዎች፡-** የበሰለ ማንጎ፡ፓፓያ፤ ጭማቂ?			
6	**ሌሎች አተክልቶች** ?			
7	**ሌሎች ፍራፍሬዎች?**			
8	**በብረት ማዕድን በለፀጉ የእንስሳት ስጋ፡-**የጉበት፤ ኩላሊት፤ልብ፤ዱለት ወይም ከደም ጋር የተቀላቀለ ሥጋ?			
9	*ሥጋ፡- የማንኛውም አንስሳ ወይም አእዋፋት ሥጋ*			**ለልጅ**
10	**ዕንቁላል፡-** የማንኛውም አዕዋፍ እንቁላል?			
11	**አሣ፡-** የአሳ ሥጋ?			
12	**ጥራጥሬዎ፡-** ከባቄላ፤ አተር፤ ምስር፤ አቾሎኒ፤ አደንጉፊ፤ ሽምብራ፤ ወይም ከነዚህ የተዘጋጀ ምግብ, ሉሎች?			
13	**ወተትና የወተት ውጤቶች፡-** አይብ, እርጎ, ወተትና ሌሎች የወተት ተዋፆዎች?			
14	**ዘይትና ቅባት ያላቸው ምግቦች፡-** ማንኛውም ከዘይት ተሰሩ ምግቦች፤ስብ፤ቅቤ?			
15	**ቀይ ተምር / ከቀይ ተምር የተዘጋጁ ምግቦች / ዘይት**			
16	**ጣፋጮች፡** ማንኛውም አይነት ስኳራማ ምግብ ወይም መጠጥ/ ስኳር የተጨመረበት?			
17	**ቅመማቅመም፤**ቡናና ሻይ፡- ማንኛውም ነገር ከቅመማቅመም፤ሻይ ወይም ቡና?			
18	**ከቤት ውጭ ስለመመገብ፡-** ትናንት ከቤተሰቡ ከቤት ውጭ የተመገበ ሰው አለ?			

ክፍል9፡- FFQ / የአንድ ምግብ የአመጋገብ ድግግሞሽ

No	Food item / የምግብ ዓይነት	Consumption/ በልተዋል 1 = Yes 0 = No	Consumption Frequency * የአመጋገብ ድግግሞሽ	Food PPn ** በምን መልክ እንደተበላ	Periods of high consumption*** በጣም የተበለበት ወቅት
1	Teff ጤፍ				
2	Wheat ስንዴ				
3	Maize / በቆሎ				
4	Sorghum ማሽላ				
5	Finger millet ዳጉሳ				
6	Barley ገብስ				
7	አጃ				
8	Potato ድንች				
9	Sweet potato / ስኳር ድንች				
10	False banana እንሰት				
11	Beans/ pea/ ባቄላ / አተር				
12	Chickpea ሽምብራ				
13	Grass peas/ ጉያ				
14	Lentils / ምስር				
15	Carrot ካሮት/				
16	Tomato /ቲማቲም/				
17	Cabbage /ጥቅል ጎመን/				
18	Kale / ጎመን				
19	Moringa አሌኮ/ሽፈራው				
20	Pepper /በርበሬ/				
21	Pumpkin /ዱባ/				
22	ቱስጣ				
23	ስናፍጭ				
24	ውሀት				
25	ስጋ/ዓሳ				

* 0 = Never
1 = Daily
2 = Two to six days/week
3 = Once weekly
4 = Once monthly
5 = Once every 3 months

** 0 = Raw
1 = Cooked
2 = Roasted
3 = As bread
4 = As Injera

***
1 = Jun-aug
2 = Sep-Nov
3 = Dec-Feb
4 = Mar-May

ክፍል 10:- Clinical assessment

A. Dental fluorosis (Dean`s Index):

Options: 1= Normal 2= Questionable 3= Very Mild 4= Mild 5= Moderate 6= Severe)

	Age	Dental fluorosis
1. Mother:	_____	_____
2. Child:	_____	_____

B. Skeletal fluorosis: Physical Exercise (1= able, 2 =unable): See picture

	Exercise1	Exercise2	Exercise3
1. Mother:	_____	_____	_____
2. Child:	_____	_____	_____

C. Symptoms of skeletal and non-skeletal fluorosis

No	Symptoms present?	1= Yes ; 0 = No	
		Mother	Child
1	Lower back pain		
2	Leg pain		
3	Arm pain		
4	Tingling sensations in the hands and feet		
5	Neck pain		
6	Muscle weakness		
7	Loss of appetite		
8	Feelings of nausea		
9	Abdominal pain		
10	Bloated feeling in stomach		
11	Polydypsia (excessive thirst)		
12	Polyuria (production of large volumes of urine)		
13	Constipation		

ክፍል11:- ከዕ/ቤት የሚሰበሰብ መረጃ /Secondary data/ Woreda office

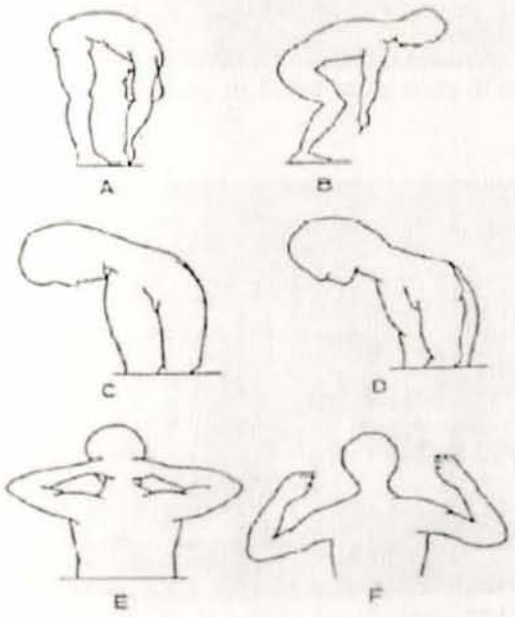
1. Mean temperature,
2. mean annual rain fall,
3. water harvesting trend,
4. total population,
5. population depending on selected water source,
6. any past attempts in mitigation of fluoride intake and fluorosis
7. future plan off the district

ክፍል12:- በውይይት የሚሰበሰብ መረጃ / FGD

1. How do people perceive fluorosis; dental and skeletal fluorosis, i.e knowledge and attitude
2. How do people/community prevent fluorosis (prevention mechanisms), efforts made so far to prevent fluorosis due to fluoride contamination
3. What are the mechanisms of mitigation of fluorosis, practices
4. What are the community dietary habits (consumed, not consumed, common diets, not allowed for all specific age/ gender
5. Annual mean temperature, maximum, minimum, when, how many months are hot, cold, medium,
6. Annual mean rain fall, how many months of rain fall, months harvested rain water used
7. food prepared in clay or stainless steel (cooking utensil)

Note for the assessment group

No	Scale (Dean`s Index)
1	Normal
2	Questionable: A few white flecks to occasional white spots.
3	Very Mild: Less than 25%. of the tooth surfaces covered by small white opaque areas.
4	Mild: Fifty per cent of the tooth surfaces covered by white opaque areas.
5	Moderate: Nearly all the tooth surfaces are involved, with minute pitting and brown or yellowish stains.
6	Severe: Smooky white appearance of all the teeth with hypoplasia, chipping and large brown stains, which vary from chocolate brown to black. There is discrete and confluent pitting, often accompanied by attrition.



Normal health individual (Exercise)

A-Can bend body and touch the floor/toes
C-Can touch chest with chin
E-Can stretch hands fold arms and touch back of head

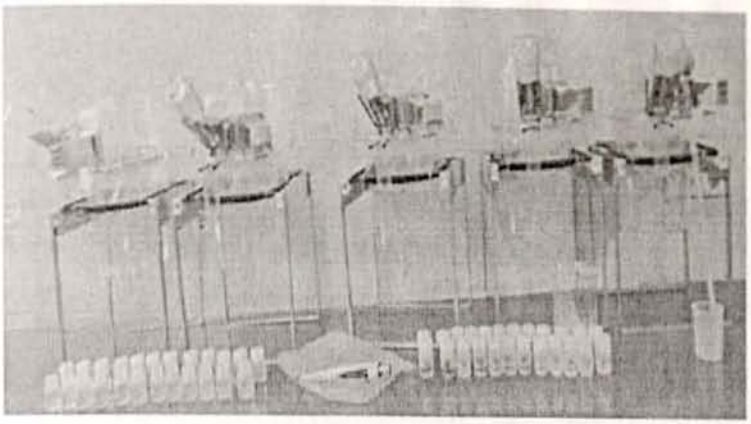
Fluoride toxicity manifestation (Exercise)

B-Unable to bend without folding knees
D-Unable to bend neck-touching chest with chin not possible
F-Unable to stretch hands, fold arms and touch back of head

7.2 Manuscripts

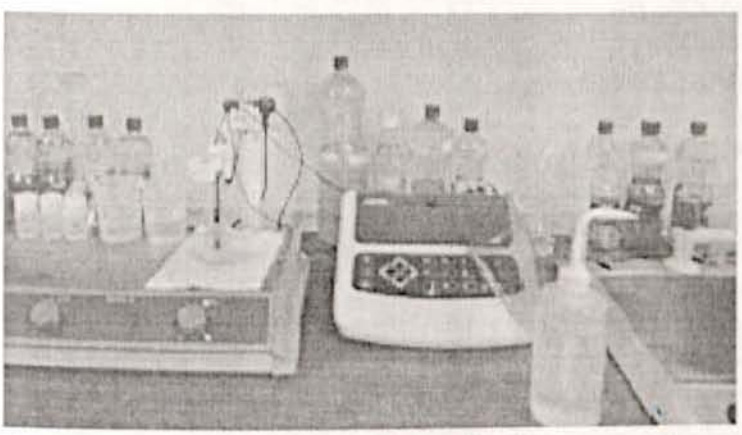
1. Dietary Fluoride sources and fluorosis risk factors in Ethiopian Rift valley
2. Community knowledge, attitude and practices (KAP) on fluorosis prevention and mitigation in endemic areas of Ethiopia
3. Dietary Fluoride Intake and Prevalence of Fluorosis in School Age Children in rural communities of Ethiopian Rift Valley
4. Potential use of calcium rich foods in mitigation of ingested fluoride

7.3 Pictures from field and aboratory work



Metabolic cage

Women drinking milk for mitigation of ingested fluoride



Fluoride analysis apparatus (Jenway 3345)

7.4 Detail of table 7

Region	District	Kebele	FoodType	F water	F food	Ca food	P food	Fe food	Z food
Afar	Gewane	Birefero	brd, wht	1.6	1.4	50.1	11.6	3.4	0.9
Afar	Amibara	Sedi Haf	brd, wht	3.6	1.1	38.1	15	4.3	1.1
Afar	Gewane	Birefero	Injera, tf + mix	1.6	2	191.8	40.6	51	3.5
Afar	Amibara	Sedi Haf	Injera, tf	3.6	1.7	53.7	12	2.3	1.1
Afar	Amibara	Awash sh	Injera, tf + mix	1.9	0.9	149.9	59.8	62.2	2.9
Afar	Amibara	Awash sh	Local brd, wht + mix	1.9	0.8	107.9	49.7	4.5	1.1
Afar	Amibara	Sedi Haf	Local brd, wht + mix	3.6	1.0	280.4	32.1	8.1	3.5
Oromiya	Bora	Botie	brd, wht	8.6	1.8	56.7	24.2	3.4	1.2
Oromiya	Boset	Cheleqil	brd, wht	9.9	1.4	55.2	11.9	9.3	1.2
Oromiya	Metahara	Merti-3	brd, wht + mix	2.8	0.8	101.7	37.1	2.9	0.9
Oromiya	Metahara	Abadir-	brd, wht + mix	4.7	2	85.8	40.3	7.1	1.2
Oromiya	Adamitul	Town	brd, wht + mix	5.2	0.9	242.5	8.5	2.6	1.5
Oromiya	Dugda	Somaya	Injera, mz + mix	6.7	2.4	100.9	32	11.9	2.1
Oromiya	Boset	Cheleqil	Injera, mz + mix	9.9	2.7	78.8	29.1	8.2	2.9
Oromiya	Boset	Borchota	Injera, mz	0.6	0.9	10	30.6	12.7	2.5
Oromiya	Adamitul	Urgomech	Injera, mz	8.7	7.8	29.4	35.8	8.2	3
Oromiya	Dugda	Tuchi	Injera, mz + mix	8.5	2.6	287	34	9.9	2.5
Oromiya	Dugda	Sariti	Injera, mz	7.2	1.5	60	59.1	9.8	2.5
Oromiya	Dugda	Bofo	Injera, tf + mix	10.8	2.5	362.2	38.1	71.8	4.3
Oromiya	Dugda	Tuchi su	Injera, tf + mix	9.5	1.7	119.5	31.7	37.8	4
Oromiya	Bora	Botie	Injera, tf + mix	8.6	2.3	310.5	39.7	18.7	4
Oromiya	Metahara	Merti-3	Injera, tf + mix	2.8	1.7	518.1	54.4	65.3	3.1
Oromiya	Adamitul	Jido	Injera, tf	5.8	2.9	22.3	37.3	32.6	3.7
Oromiya	Adamitul	Town	Injera, tf + mix	5.2	1.3	142.6	43	25.2	3.9
Oromiya	Adamitul	Anano	Injera, tf + mix	5.4	1.6	109.1	54.9	24.6	9
Oromiya	Metahara	Abadir-	Injera, tf + mix	4.7	1.8	401.2	48.3	31.8	2.5
Oromiya	Dugda	Bofo	Injera, tf + mix	9	-	119.2	27.9	23.3	3.7
Oromiya	Dugda	Orgocho	Injera, tf + mix	7.9	2	239.9	44.1	14.5	2.4
Oromiya	Metahara	Northcam	Injera, tf + mix	2.2	2	106.4	37.5	38.5	2.9
Oromiya	Bora	Tejitu	Injera, tf + mix	8.2	2.4	672.1	46.1	40.8	4.4
Oromiya	Dugda	Tuchi	Injera, tf + mix	7.2	1.8	248.4	42.8	33.3	6.1
Oromiya	Bora	Dalota	Injera, tf + mix	4.8	2.2	250.7	48.9	28	4
Oromiya	Dugda	Bofo	Local brd, wht + mix	10.8	1.7	131.1	33	8.8	3.1
Oromiya	Metahara	Merti-3	Local brd, wht + mix	2.8	1.2	248.4	55.8	4.8	1.5
Oromiya	Adamitul	Anano	Local brd,mz	5.4	1.3	19.3	42.1	3.8	2.1
Oromiya	Adamitul	Dodecha	Local brd,mz	1.9	1.4	30.2	55.5	4.4	2.4
Oromiya	Boset	Borchota	Local brd,mz,wht	0.6	4.4	38.5	39.7	10.3	2.5
Oromiya	Adamitul	Gorbii	Local brd,mz,wht	4.6	1.5	42.3	40.6	8.3	3.8
Oromiya	Dugda	Tuchi su	Local brd,wht	9.5	1.7	27.1	42	10.2	4.1
Oromiya	Metahara	Northcam	Local brd,wht	2.2	-!	44.6	39.7	4.3	2.8

Region	District	Kebele	FoodType	F water	F food	Ca food	P food	Fe food	Z food
Oromiya	Adamitul	Jido	Local brd,wht	5.8	7.7	20.4	30.2	10.9	3.8
Oromiya	Dugda	Orgocho	unleavened brd,mz	7.9	1.3	49.4	152.3	14.2	2.2
Oromiya	Boset	Borchota	unleavened brd,mz	0.6	0.9	45.6	30.1	18.1	2.3
Oromiya	Metahara	Northcam	unleavened brd,mz	2.2	0.8	57	43.7	5	2
Oromiya	Adamitul	Mechefer	unleavened brd,mz	0	2.6	23.1	43.2	4.9	2.4
Oromiya	Metahara	Abadir-	unleavened brd,milet	4.7	1.6	232.1	69.8	7.3	1.3
Oromiya	Adamitul	Anano	unleavened brd,mz	5.4	3.3	23.9	21.1	4.9	2.4
Oromiya	Adamitul	Dodecha	unleavened brd,mz	1.9	6.4	93	52.8	3.4	3
Oromiya	Adamitul	Jido	unleavened brd,mz	5.8	1.8	22.1	33.9	8.2	2.5
Oromiya	Adamitul	Urgomech	unleavened brd,mz	8.7	1.7	30.9	36.4	3.6	1.9
Oromiya	Adamitul	Gorbii	unleavened brd,mz	4.6	1.3	8.1	24.8	6	2.3
Oromiya	Adamitul	Merisa	unleavened brd,milet	6.3	1.9	258.2	51.4	5.4	2.4
Oromiya	Dugda	Sariti	unleavened brd,mz	7.2	1.3	39.6	52.4	7.1	2.6
SNNPR	Measkan	Bechegul	Injera, mz	3.6	1.8	51	36.6	8.5	2.7
SNNPR	Meskan	Bechegul	Injera, mz	3.4	0.8	41.6	60.5	5.9	2.3
SNNPR	Meskan	Bechegul	Injera, mz	4.2	1.7	51.5	78.5	12	2.6
SNNPR	Wolayita	Town	Injera, tf + mix	9.5	7.3	124.4	34.9	30.9	5
SNNPR	Alaba	Bishano	Injera, tf + mix	3.8	2.6	296.5	58.3	43	3.2
SNNPR	Mareko	Elalo ji	Injera, tf	2.9	1.3	45.1	53.3	15.9	3.2
SNNPR	Mareko	Hobejard	Injera, tf	1.3	1.1	85.3	249.4	10	2.7
SNNPR	Mareko	Faqowara	Injera, tfrv,mz	3.6	2.1	89.4	52.2	62.5	3
SNNPR	Meskan	Bechegul	Injera, tfrv,mz	2.2	1.6	62.5	16.1	24.1	3.1
SNNPR	Mareko	Elalagab	Injera, tfrv,sgm	6.5	1.6	40.5	34.3	25.1	3.3
SNNPR	Alaba	Uppertuq	Injera, tf + mix	3.4	1.4	219.1	38.3	63.5	2.9
SNNPR	Mareko	Elalagab	Local brd,mz	6.5	1.5	27.5	38	8.8	2.7
SNNPR	Measkan	Bechegul	Local brd,mz	3.6	2	34.6	40.7	5	2.3
SNNPR	Meskan	Bechegul	Local brd,mz	3.4	1.6	63.3	36.2	8.5	2.5
SNNPR	Mareko	Faqowara	Local brd,mz	3.6	0.9	46.8	76.3	14.4	3.2
SNNPR	Meskan	Uchegena	Local brd,mz	0.8	0.6	79.4	60.7	9.2	2.9
SNNPR	Mareko	Elalo ji	Local brd,mz	2.9	1.5	18.1	55.7	9.7	2.3
SNNPR	Meskan	Bechegul	Local brd,mz	2.2	1.3	26.8	25.5	7.9	3
SNNPR	Mareko	Hobejard	Local brd,mz	1.3	1.1	43.7	270.1	8.9	2.2
SNNPR	Meskan	Bechegul	Local brd,mz	4.2	1	39.6	44.9	9.2	2.3
SNNPR	Wolayita	Town	Local brd,mz,wht	9.5	2.6	21.4	17.9	2	1.3
SNNPR	Alaba	Bishano	unleavened brd,mz	3.8	1.4	46.7	37.3	3	2
SNNPR	Mareko	Faqowara	unleavened brd,mz	3.6	1.2	22.9	18.6	10	2.9
SNNPR	Wolayita	Town	unleavened brd,mz	9.5	1.5	52.4	349.9	3.3	2.6