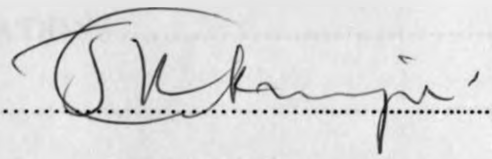
DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.



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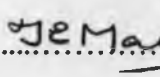
J. K. GIKUNJU

This thesis has been submitted for examination with our approval as University supervisors.



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Prof. Timothy E. Maitho, B. V. M., M. Sc., Ph.D.

Department of Public Health, Pharmacology and Toxicology,

University of Nairobi

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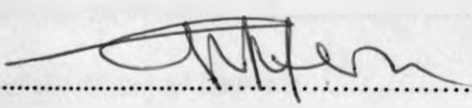
Prof. Eric S. O. Mitema, B. V. M., M. S., Ph.D.

Department of Public Health, Pharmacology and Toxicology,

University of Nairobi



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Prof. Gerald M. Mugera, Dip. Vet. Sc., M. Sc., Ph.D, D. Sc.

Department of Veterinary pathology and Microbiology,

University of Nairobi

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LIST OF ABBREVIATIONS

BDH	British drug house
TISAB III	Total ionic strength adjustment buffer III
PPM	Part per million
WHO	World Health Organisation
S.D	Standard Deviation
ANOVA	Analysis of Variance
Ca	Calcium
P	Phosphorus
Mg	Magnesium
F	Fluoride
HF	Hydrogen fluoride
TDI	Total daily intake
MFP	Monofluorophosphate
NRC	National Research Council
CaF	Calcium fluoride
CL	Chloride
K	potassium
SiF_6	Fluorosilicate
Fe	Iron
Pb	Lead
Cu	Copper
So_4	sulphate

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DEDICATION

This work is dedicated to my wife, Irene and my children Gideon, Sharon and Joy for their co-operation, encouragement and understanding during the course of my study.

God is good all the time and all the time God is good.

ABSTRACT

Excessive ingestion of fluoride can cause dental and skeletal lesions and in severe circumstances adversely affect health and productivity performance of domestic animals. The objectives of this study were to investigate fluoride dietary sources, effects of fluoride on milk production, excretion of fluoride in cow milk and urine and fluoride toxicity in rats. Fluoride concentration was determined using fluoride ion specific electrode and the mean recovery percentages were : 92.4 ± 7.7 % (n=114).

One hundred and four samples of feedstuffs and 149 water samples for dairy cattle were collected from six dairy co-operative societies within Kiambu and Thika districts of Kenya during the wet and dry seasons of the year1994 in a cross-sectional study. The mean fluoride concentration in feeds from Nderi, Kikuyu, Chania, Limuru, Kiambaa and Lari co-operative societies were :19.5 ± 11.3 (n=19) 24.1 ± 28.6 (n=22), 55.2 ± 73.7 (n = 18), 67.6 ± 93.4 (n=15), 91.9 ± 226.3 (n=24) and 203.4 ± 243.2 (n=6) mgF/kg, respectively. Individual dairy co-operative society and the type of sample significantly ($p < 0.05$) influenced fluoride concentration in feedstuffs. The overall mean fluoride concentration in water was 0.25 ± 0.45 mg/L (n=149). The fluoride concentration in water during the dry season were significantly ($p < 0.05$) different from fluoride concentration during the wet season.

One hundred and thirty dairy milk and 106 urine samples of dairy cattle were obtained for fluoride analysis. The mean fluoride level in milk was 0.066 ± 0.14 mg F/kg while mean fluoride concentration in cows' urine was

1.28 ± 1.0 mg F/kg. Seasons, breed of cattle, source of water and dairy co-operative society did not affect milk and urine fluoride concentration significantly ($p > 0.05$).

Two hundred and forty six dairy cows were assessed for milk production. The mean milk production was 3.13 ± 2.78 l/cow/day. There was a significant difference ($p < 0.05$) in milk production due to season and dairy co-operative society. Water and feedstuff fluoride concentration did not significantly influence ($p > 0.05$) milk production.

The toxic effects of fluoride in female wistar rats (n=100) were investigated within a time-span of 96 to 843 days. Rats were randomly divided into 10 groups of 10 rats per group namely A, B, C, D, E, F, G, H, I and J. Each group was provided with commercial rat feed of known fluoride content and graded doses of fluoride in de-ionised water. Groups A, B, C, D, E and F were fed on 1, 5, 10, 30, 60, 80 mg F L^{-1} equivalent to 0.087, 0.42, 0.823, 2.667, 5.45 and 7.804 mg/kg sodium fluoride in de-ionised water respectively. Group G, H, I and J were fed on 2 % Magadi salt solution, de-ionised water (control), 2 % commercial mineral salt solution and 2 % tea extract respectively. The following variables were monitored for each group; bodyweight, organ weights, water (fluoride) intake, feed intake, fluoride concentration in tissues and fluoride concentration in faeces. Sixty-two rats were sacrificed during the time of the experiment for tissue fluoride assays while twenty-eight rats were killed for pathology tissue processing. Ten rats died in the course of the experiment and were dissected

for fluoride tissue assay and pathology. The dose of fluoride in drinking water significantly influenced ($p<0.05$) body weight, tissue fluoride concentration and organ weight. Rats fed on magadi salts had the lowest mean weight (186.7 ± 18.8 g) as compared to the control group of rats which had a mean weight of 280.0 ± 17.1 g whereas group C had the highest mean weight of 343.7 ± 40.7 g.

Histopathological examination on liver, kidney, lungs and the heart organs of rats from groups A, B, C, H, and J revealed no pathological changes, however in groups D, E, F, G and I, degenerative changes, hepatic and myocardial haemorrhages were observed. The pathological changes became more severe as the concentration of fluoride was increased. Tumour growths were observed in three rats: uterine adenocarcinomas (group A and group C) and a fibroma (group J). One control rat from group H had a pyogranulomatous nodule as well.

Fluoride concentration in the muscle, femur, incisor teeth and lower jaw were: 19.0 ± 28, 693.9 ± 536.4, 730.5 ± 576.8 and 1063.5 ± 829.6 mg /kg respectively. Fluoride concentration in lower jaw were significantly higher ($p<0.05$) than in muscle tissues. Faecal fluoride excretion was significantly influenced ($p<0.05$) by time of fluoride exposure.

Nine food substances (Tilapia, Nile perch, lettuce, spinach, bovine, cabbage, kales, goat and chicken) were obtained from the local market and used to prepare fresh soups. Fluoride concentration in ninety fresh soup samples were investigated upon boiling for two hours. Soup samples were

drawn from each preparation at intervals of 15 minutes. Tap water was used as a control. The type of food substance used for soup preparation and boiling significantly influenced fluoride concentration in the soup. Fluoride concentration in Tilapia, Nile perch, lettuce, spinach, bovine, cabbage, kales, goat, and chicken soup were: 5.01 ± 1.27, 2.92 ± 0.54, 0.67 ± 0.34, 0.66 ± 0.28, 0.32 ± 0.05, 0.25 ± 0.05, 0.24 ± 0.03, 0.22 ± 0.04 and 0.16 ± 0.04 ppm respectively. Soup prepared from Tilapia fish had significantly higher, ($p < 0.05$) fluoride concentration than other meat and vegetable soups.

This cross -sectional study has shown that dairy cattle from Kiambu and Thika districts are exposed to high concentration of fluoride especially through mineral supplements. Consequently, there is need to establish standards for fluoride in mineral mixes and other animal feeds. In addition this study has shown that fluoride is essential for normal growth of rats and the optimal fluoride intake in drinking water is 10 ppm. Further, the analytical method used in this study was found suitable for determination of fluoride in water, soup, food substances and animal feeds.

Overcooking may increase fluoride concentration in Tilapia soup and further investigation should be carried out on bioavailability of fluoride from fish soup. In addition further investigation should be done to establish whether fluoride is tumorigenic in rats.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Introduction

Minerals originate from the earth's crust and make up only a small proportion of the body tissue. Minerals are essential for building body tissues and regulating body processes. Certain inorganic elements including calcium (Ca), phosphorus (P) magnesium (Mg) and fluoride (F) are components of bones and teeth. Fluoride is a component of bones and teeth. Little significance was attached to fluoride as a toxic agent until it was shown that continued ingestion of small quantities of fluoride produces chronic poisoning. It is now established that excessive fluoride concentration in the soil or water may lead to chronic endemic fluorosis. The most visible signs of fluorosis include discolouration of the teeth in man and lameness in cattle.

Excessive fluoride ingestion can cause dental and skeletal lesions and in some cases adversely affect the health and productivity of domestic animals (Shupe and Olson, 1971; Suttie, 1983; Bunce, 1985, Wheeler and Brock, 1985 and Krook 1998). Fluoride intake exceeding 5 parts per million (ppm) has adverse effects on reproduction in cows (Rensburg and Vos 1966). High intake of fluoride has been associated with infertility in rats (Zhao and Wu 1995), rabbits (Susheela and Kumar, 1991) and man (Freni, 1994). In Kenya, fluorosis in farm animals is often a problem in volcanic areas especially in animals drinking from deep boreholes (Said, 1981). This applies mainly to the arid zones in the Kenyan Rift Valley and adjacent areas. Fluoride intoxication

caused loss of body condition and a fall in milk production in a herd of dairy cattle in Kenya (Murray, 1967). Fluoride concentration in fish and water from Kenyan lakes are high and may contribute to the high prevalence of dental fluorosis in Kenya, (Gikunju *et al* ., 1992). Information is scarce on fluoride toxicity in animals in Kenya (Murray, 1967, Said, 1981).

Commercial feed concentrates and mineral mixture with excessive amounts of fluoride cause low milk production (Eckerlin, *at al.,* 1986), while long term administration of fluoride at 2.15 ppm in drinking water can cause fluorosis in dairy cows and decrease milk production (Xiao and Zhu, 1987). In Kenya, data is lacking on fluoride consumption in dairy animals hence there is a need to carry out a detailed fluoride investigation in the country.

Dogs, cats and horses also suffer from fluorosis mainly due to ingestion of food containing high concentration of fluoride (Shupe and Olson, 1971, Mumma *et al.,* 1986). Wild animals are susceptible to adverse effects of excessive ingestion of fluoride and primary effects are observed on teeth and bone lesions (Shupe *et al.,* 1984).

The geology of Kenya renders it one of the places in the world, where fluoride occurs in high concentrations (Williamson, 1953, Manji and Kapila, 1984, and Gaciri and Davies, 1993). Hence, endemic dental fluorosis is widespread, and is a serious public health problem in Kenya (Gitonga and Nair 1982). High fluoride concentrations have also been found in raw materials of animal feed, mostly of marine origin (Trautner and Siebert 1985). This fluoride might be further concentrated in the food chain and pass into

products for human consumption. There is currently no guidelines on the concentration of fluoride acceptable in various animal feeds and water consumed on a daily basis in Kenya and therefore more studies are required on fluoride consumption and toxicity. The tolerance concentration of fluoride in drinking water for dairy cattle is 3 to 6 mg/L and 30 to 40 mg/kg in feeds (National Research Council (NRC), 1994). However, these values do not fit the Kenyan situation due to climatic and physiological factors. Although the toxicity of sodium fluoride in rats has been reported by Shourie *et al.*, (1950), Whitford *et al.*, (1987), Gruninger *et al.*, (1988) Dote *et al.*, (1998) and, Usuda *et al.*, (1998) more investigation is required. This study was conducted in order to investigate fluoride dietary sources, effects of fluoride on milk production, excretion of fluoride in cow milk and urine and its toxicity in rats. The study will also provide data which may be necessary for development of guidelines on fluoride exposure.

1.2 OBJECTIVES

The specific objectives of the study were:

I) To standardise fluoride assay and determine fluoride concentration in fresh soup prepared from meat, fish and vegetables,

II) To determine fluoride concentration in dairy cattle feedstuffs, water, milk and urine during the dry and wet seasons,

III) To establish relationship between dietary fluoride concentration and milk production,

IV) To investigate fluoride toxicity in female wistar rats.

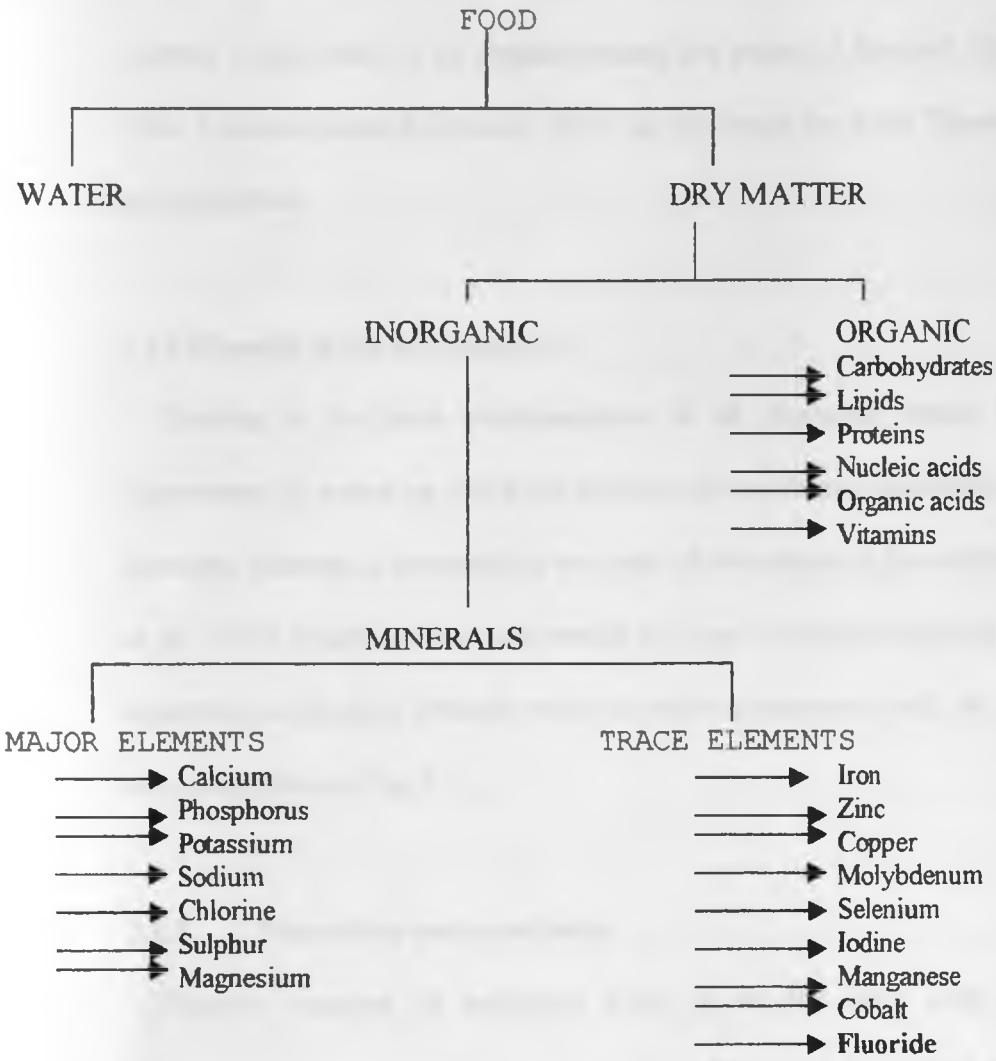
CHAPTER TWO

LITERATURE REVIEW

2.0 INTRODUCTION

Mineral elements are classified into two groups on the basis of relative amounts in the body. Minerals occurring in relatively large amounts and are required in quantities of over 100 mg per day are called macrominerals (Fig. 2.1,) and include: calcium, chloride, magnesium, phosporus, potassium and sodium. Minerals occurring in small amounts and are required in quantities of a few mg or less per day are called microminerals or trace minerals (Tables 2.1 and 2.2,) and include : chromium, cobalt, copper, fluoride, iodine, iron, manganese, molybdenum, selenium and zinc. Over 30 other minerals such as lead, gold and mercury also occur in the body tissues. They are potentially harmful and originate mainly from environmental pollution. Some minerals are part of hormones, enzymes, and other compounds which regulate body functions. For instance, iodine is required to produce thyroxin hormone, chromium is involved in production of insulin, calcium is a blood clotting factor (IV) and haemoglobin is an iron containing compound. Some minerals catalyse gastrointestinal absorption of nutrients, metabolism of proteins , carbohydrates and fats (McDonald, 1992).

Fig.2.1. The main components of food - animals and plants



Source: McDonald, 1992.

The terms "fluorine" and "fluoride" are often used interchangeably when referring to compounds of fluorine, however "fluorine" is defined as "a gaseous chemical element", whereas "fluoride" is defined as "a compound of fluorine with a metal, a non metal, or an organic radical; the anion of fluorine" (Shupe *et al.*, 1963, National research Council, 1971). In this thesis the word "fluoride" is used as defined here.

2.1.1 Fluoride in the environment

Fluorine is the most electronegative of all elements, hence it is rarely encountered in nature as elemental fluorine but combines chemically with other elements. Fluorine is thirteenth in the order of abundance in the earth's crust (Bell *et al.*, 1970). Fluoride occurs universally and may be either beneficial or harmful, depending on the dose. Fluoride occurs in various amounts in soil, air, water, plant and animal tissues (Fig. 2.1).

2.1.2 Fluoride in rocks and soils

Fluorine occupies an estimated 0.065 % of the earth crust by weight. Compounds whose molecules contain atoms of fluorine are widely distributed in nature particularly in sedimentary and igneous rocks. Deposits of fluorspar (calcium fluoride, CaF2), the chief ore, are found in many parts of the world, including Kerio Valley in Kenya. Cryolite, (Na_3AlF_6), which is used both in the ceramic industries and the metallurgy of aluminium, is found in only a few places. Fluoroapatite, ($Ca_5F(PO_4)_3$), frequently called rock phosphate, is the most plentiful

mineral (Moller, 1982). Fluoride occurs most commonly as fluorite or fluorspar (CaF_2), which may contain upto 49 % fluoride.

Fluoride in soils is derived from the geologic parent material. The water-soluble fluoride is of interest since it may affect plant and animal life. Volcanic eruptions contribute large amounts of fluoride to surface soils by way of ash deposited on the terrain. Annual addition of fluoride containing superphoshate fertilisers increases the levels of fluoride in the soil. The occurrences of fluoride in the various types of rocks composing the earth crust have been reviewed by Correns (1956).

2.1.3 Fluoride in Water

Fluoride enters the water cycle by leaching from soils and minerals into ground water and surface water. Fluoride concentration in water is affected by several factors such as availability and solubility of fluoride containing minerals, porosity of the rocks or soil through which the water passes, temperature, pH and the presence of other elements which may complex with fluoride (Fleisher and Robinson, 1963). The fluoride concentrations in Kenyan water is higher than that reported from any other country in the world. The highest concentrations of fluoride in Kenyan water occurs in water from some springs, boreholes and in some lakes in the Rift Valley (World Health Organisation) WHO, 1970, 1973; Gitonga and Nair, 1982; Manji and Kapila, 1984; Gikunju *et al.*, 1992, Mwaniki and Gikunju, 1995).

Natural compounds containing fluorides are only sparingly soluble, therefore surface waters do not usually have high fluoride levels. The recommended levels

of fluoride in water are 0.7 and 1 ppm for a hot and temperate climate respectively (McCLure, 1970). However, dental fluorosis has been reported in communities supplied with fluorinated and non fluorinated water in U. S. A. Similarly, dental fluorosis has been reported in Kenya in areas of low fluoride in water (Manji, *et al.*, 1988). This implies that sources of fluoride other than drinking water might be of importance in contributing to the total daily fluoride intake particularly in such areas with low fluoride in water.

2.1.4 The Chemistry of fluoride

The term fluorine was derived from "fluere" which is a latin word meaning "to flow". Fluorine has an atomic weight of 18.998 although there are two other radioisotopes of atomic weight 17 and 22. It has an electronic configuration of $1s^2$ $2s^2$ $2p^5$. Fluorine was first isolated in 1886 by the French chemist Moissan, who applied a method originally suggested and later tried by Davey and Ampere in 1810-1812. Moissan's success was due to the use of a dilute solution of potassium fluoride in completely anhydrous hydrogen fluoride. Fluorine forms hydrofluoric acid, (HF) in water and oxygen difluoride (OF_2) with oxygen. Under controlled conditions, fluorine reacts with sodium hydroxide to form a soluble salt sodium fluoride (NaF), oxygen and water. This reaction is utilised for the disposal of fluorine by conversion to a soluble salt.

When a fluoride compound is dissolved in water, the element fluorine will be present mainly as fluoride ion, (F^-). However, depending on the ionic concentration and the pH of the solution, the fluoride will also be present as undissociated

hydrogen fluoride (HF). In dilute solutions and at neutral pH, virtually all the fluoride will be present as fluoride ion, F^-. However, as the pH of the solution decreases, the proportion of F^- present decreases while the proportion of undissociated HF increases (Borei, 1945).

2.1.5 Defluoridation of portable water

Defluoridation of water is a relatively poorly developed process when compared to other water treatment processes like filtration, coagulation, flocculation, sedimentation and disinfection. Consequently very little has been done on defluoridation of public water supplies with an effective economical reliable and universally accepted method in Kenya, (Gitonga and Nair, 1982; Mwaniki and Gikunju 1995). The most obvious way of reducing exposure to water-borne fluoride is to change the water supply to one with acceptable fluoride levels. A brief review of Physico-chemical removal of fluoride is presented below.

2.1.5.1 Activated carbon

This is produced from heated and ground wood, puddy husks, and coconut fibre, etc. It is most effective when the pH is low but this is disadvantageous because pH of water has to be raised to make it acceptable for consumption.

2.1.5.2 Bone

Dried and crushed bone and bone char (dried and crushed bone heated to 600 °C for 20 minutes) are efficient removers of fluoride. Bone char is preferred because

bacteria contamination is reduced and taste is improved compared with bone. Bone char can be regenerated with caustic soda. However, some religions do not accept bone as a defluoridation agent. A 3: 1 mixture of bone char and charcoal has been used in Thailand (Phantumvanit, *et al.*, 1988) and may be especially useful for household units. Although bone char has been recommended in the defluoridation of drinking water in developing countries, parameters relating to fluoride sorption characteristics by type of bone char are unclear. However, black bone char is reported to be more efficacious than grey and white bone char in partial defluoridation of drinking water, (Mwaniki 1992).

2.1.5.3 Lime and Aluminium sulphates

They have a high affinity for fluoride and may be used for defluoridation. Some of the disadvantages include pH and alkalinity control, hardness is removed together with fluoride and aluminium poisoning may occur. The combined use of lime and aluminium is the central feature of the Nalgoda technique developed at the Indian National Environmental Engineering Research Institute at Nagpur in 1974 (Buluyu 1988). It was developed for medium sized communities but is adaptable to village level. Magnesium oxide can be used as an alternate to calcium oxide. Aluminium chloride is sometimes used together with aluminium sulphate.

2.1.5.4 Magnesium oxide and bone meal

Opinya, *et al.*, (1987) investigated the possibility of using magnesium oxide or bone meal to defluoridate Kenyan drinking water. Both removed fluoride but

reaction time was faster with bone meal than with magnesium oxide and the taste of water treated with bone meal was better. Both magnesium oxide and bone meal are readily available and inexpensive in Kenya.

2.1.5.5 Other techniques

Ion-exchange resins are commercially produced for removal of fluoride e.g. Polystyrene resins, defluoron 1 and 2. They are expensive and treated water has a poor taste. Activated alumina (aluminium oxide) has been used in U. S. A. and India. Reverse osmosis and electrolysis is an effective defluoridation technique but it is expensive.

2.1.6 Fluoride in the Atmosphere

Fluoride enters the atmosphere by volcanic action and by entrainment of soil and water due to the action of wind on the surfaces. It is returned to the earth's surface by deposition as dust or in rain. Additional fluorides are widely distributed in the atmosphere originating from the dusts of fluoride-containing soils (Williamson, 1953), gaseous industrial wastes, the burning of coal fires in populated areas (Cholak, 1959), and from the gases emitted in areas of volcanic activity. All these sources increase the fluoride level of rain or precipitation. Hazards to crops, animals and human health caused by atmospheric fluorides are well documented (Largent, 1961; Hodge and Smith 1965; Thomas and Alther 1966; Agricultural Research Council, 1967;Vostal 1971; Vikoren 1995).

2.1.7 Occurrence of fluoride in Vegetation

The widespread prevalence of fluoride in soil, water and rocks result in the presence of fluoride in many plant tissues. However, it is known that the fluoride content of most plants, with the exception of the roots, is not readily affected by the amount of fluoride in the soil in which they grow. Exception to this general rule are the *Camelia spp* and tea plant. Figures for the former have been reported to be upto 2000 ppm and for the latter, 150 ppm (Allcroft *et al.*, 1965). Plants generally have limited ability to accumulate fluoride from soils, although acidic soils can enhance uptake (Underwood, 1977). Grains have low fluoride concentration, ranging from 1 to 3 ppm (Underwood, 1977).

Fluorine from the atmosphere combines with water and particles in the air eventually settling on vegetation consumed by livestock. Forages and silage are most at risk of being contaminated by fluoride from aluminium smelters, steel mills, or fertilizer plants.

Organic forms of fluoride (fluoroacetate and fluorocitrate) are often formed in some forage and grain crops grown in areas contaminated with atmospheric fluoride. Sodium fluoroacetate (Compound 1080) and fluoroacetamide (compound 1081) were used as rodenticides in the past. Compound 1080 was banned by the Environmental Protection Agency in 1972 because of its dangers, but intensive lobbying efforts by the livestock industry resulted in the lifting of this ban in 1985. Livestock Protection Collar (LPC), which is used by the livestock industry is fitted on livestock and contains Compound 1080. The poison is intended to leak out and kill a predator during a direct attack on livestock (Timbrell 1987).

Monofluoroacetic acid (fluoracetate) is a compound found in certain African plants, for instance *Dicapetulum cymosum* and causes severe toxicity in animals eating such plants. Fluoracetate does not cause direct tissue damage but requires metabolism to fluoroacetyl CoA, which is incorporated into the tricarboxylic acid cycle analogous to acetyl CoA (Timbrell, 1987). Fluoracetyl CoA combines with oxaloacetate to form fluorocitrate which inhibit aconitase enzyme leading to build up of fluorocitrate and citrate. Toxicity occurs due to malfunction of the central nervous system and heart leading to nausea, apprehension, convulsions, arrhythmias and ventricular fibrillations. Fluoroacetate and fluorocitrate do not inhibit other enzymes in the TCA cycle (Timbrell, 1987).

2.2.0 FLUORIDE PHARMACOKINETICS

Hydrogen fluoride (HF) is a weak acid with a pKa of 3.4. There is evidence showing that fluoride absorption is pH dependent and transmembrane migration of the ion occurs in the form of HF in response to differences in the acidity of adjacent body fluid compartments (Whitford, 1989). Studies with lipid bi-layer membranes showed that the permeability coefficient of HF is more than one million times greater than that of ion fluoride (Gutknecht and Walter, 1981).

After ingestion, plasma fluoride levels increase within few minutes and reach a maximum concentration after 20 minutes. The maximum concentration depends on the amount ingested, rate of absorption, volume of distribution and the rates of fluoride clearance from plasma by the kidneys and the skeleton. The rapid decline

in plasma concentration that occurs after the rate of absorption declines is due to the renal and skeletal clearances of fluoride, (Whitford 1994).

2.2.1 Absorption of fluoride

The absorption, soft tissue distribution, calcified tissue uptake and renal excretion of fluoride occur simultaneously. A pharmacokinetic analysis of the plasma fluoride concentration curve defines various processes and kinetics of fluoride. The phases which are readily distinguished include: absorption, distribution (alpha phase) and elimination (beta phase), and can be described using pharmacokinetic models. Fluoride is rapidly distributed in the well perfused tissues such as the heart, kidney, liver, and also to the bone since fluoride is a calcified tissue-seeker. It is more slowly distributed to poorly perfused tissues like skeletal muscles and adipose tissues. The pharmacokinetics of fluoride follow first order kinetics, where the rate of elimination is directly proportional to the plasma fluoride concentration (Ekstrand and Whitford 1988).

Fluoride is absorbed from the gastrointestinal tract, the lungs, and the skin. The absorption of fluoride from the gut is influenced by anatomical, physiological and biochemical factors. The greater gastrointestinal absorption of fluoride in the rabbit than in the rat is attributed to the longer gastrointestinal tract of the rabbit. In ruminants, fluoride absorption is mainly confined to the rumen (Perkinson, *et al.*, 1955).The degree of absorption of a fluoride compound is correlated with its solubility. The relatively soluble sodium fluoride, is almost completely absorbed, whereas the relatively insoluble compounds, such as cryolite (Na_3AlF_6) and the

fluoride found in bone meal (fluoroapatite), are poorly absorbed. Certain cations (e.g. aluminium, calcium and iron) retard the absorption of the fluoride ion by forming low-solubility complexes in the gastrointestinal tract and faecal excretion increases (Hodge and Smith, 1965).

The second most important route of absorption is by way of the lungs. Pulmonary inhalation of fluoride present in dusts and gases constitute the major route of industrial exposure. A third, and relatively rare, route of exposure, is through the skin. Fluoride from various sources may be absorbed at different rates.

Soluble fluoride compounds added to normal human diets are readily absorbed when they are added to water, whereas the absorption of less soluble fluorides included in food may be reduced by 20 %. The poor availability of fluoride from bone meal and sardines is due to their high calcium content. Only part of the fluoride chemically bound to calcium is set free by digestive processes. Jowsey and Riggs (1978) reported a less increase in plasma fluoride values after concurrent administration of calcium carbonate and sodium fluoride than after sodium fluoride alone. Patz *et al.*, (1977), and Afseth, *et al.*, (1985), found no increase in plasma fluoride level following ingestion of calcium fluoride tablets despite their high solubility *in vivo* 0.1 M Hyrochloric acid. In contrast, Spencer *et al.*, (1981), observed no change in fluoride absorption in man when several doses of calcium gluconate ranging from 200 to 2000 mg calcium were given daily. However, the intake of fluoride was separate from that of calcium gluconate. The influence of concurrent administration of fluoride and calcium in the absorption of fluoride was shown by Quaassdorff (1985), who fed rats a normal diet with 1.1 %

calcium and 30 to 80 mg F/kg diet and observed fluoride absorption of 36 %. This absorption rate increased to 85 - 94 % when fluoride was given separately from the calcium containing diet, by use of a feeding machine.

The delay in fluoride uptake from milk cannot be fully explained by the formation of calcium fluoride with milk calcium (Ekstrand and Ehrnebo, 1979). There are several mechanisms which may influence fluoride release and absorption from food. Greater food intake leads to increased acid production in the stomach which increase fluoride absorption (Whitford and Pashley, 1984). The quantity of food influences the speed of emptying of the stomach and thus the period of fluoride absorption in the upper part of the gastrointestinal tract. Concurrent intake of food influences fluoride absorption, although the mechanism is not fully understood (Trautner and Siebert, 1986).

In the absence of high concentrations of certain cations, such as calcium and aluminium, which form insoluble componds with fluoride, about 80 to 90 % of the ingested amount is absorbed from the gastrointestinal tract (Cremer and Buttner, 1970). The half-life for absorption is 30 minutes. The rate of gastric absorption is directly related to the acidity of the contents and, the peak plasma level is higher and occurs sooner when the contents are more acidic (Whitford and Pashley, 1984). Most of the fluoride that escapes absorption from the stomach is absorbed from the proximal part of the small intestines (Whitford, 1989). Fecal fluoride excretion in rats can be increased significantly by elevating plasma fluoride concentration and increasing the calcium concentration of the diet (Whitford and Augeri, 1993; Whitford 1994).

2.2.2 Distribution of fluoride in the body

Fluoride has been detected in all organs and tissues examined, however, there is no evidence indicating that it is concentrated in any tissue except skeletal tissues, and to a lesser extent in the thyroid, aorta and kidney. Fluoride is deposited in the bones and teeth, and the degree of skeletal storage is related to intake and age. This is thought to be a function of the turnover rate of skeletal components, with growing bone showing a greater deposition than bone of mature animals. Prolonged period of time is required for mobilization of fluoride from bones. Fluoride is accumulated by the aorta, and concentrations increase with age, probably reflecting the calcification that occurs in this artery. Approximately 99 % of all the fluoride in the body is found in calcified tissues (Ekstrand and Whitfold, 1988). The selective affinity of fluoride for mineralised tissue is due to uptake of fluoride by the process of iso-ionic and heteroionic exchange leading to incorporation into the bone crystal lattice structure as fluoroapatite or fluorohydroxyapatite. Fluoride is not irreversibly bound to bone. In the foetus, fluoride is readily taken up by the calcifying foetal bones and teeth. Normal plasma fluoride levels in man range from 0.7 to 2.4 ppm. Plasma fluoride concentrations are not homeostatically regulated (Whitford and Williams, 1988), but fluctuates according to fluoride intake, and other factors such as the rate of bone resolution and the fluoride renal clearance. There is a direct relationship between the fluoride concentration in bone and in the plasma.

2.2.3 Fluoride in the placenta

Studies conducted in several species have shown that fluoride crosses the placenta and is taken up by fetal tissues in limited amounts (Flatla and Ender, 1967; Bell *et al.*, 1970; Parker and Bawden, 1986; Gupta *et al.*, 1993). The bone fluoride level in newborn calves is correlated with the amount ingested by the dam and her blood fluoride concentration. The human placenta acts as a barrier to fluoride diffusion when the fluoride concentration in maternal blood exceed 0.4 ppm, (Gupta *et al.*, 1993). The degree to which the placenta acts as a barrier vary from one species to the other due to differences in the nature of placenta (Shupe and Olson, 1983; Parker and Bawden 1986). It is still unclear whether the amount of fluoride transferred from the dam to the fetus is high enough to cause adverse effects.

2.2.4 Fluoride in the plasma

Fluoride in plasma is not bound by proteins or any other constituent of plasma (Taves, 1968). The concentration varies according to the level of intake and physiological factors (Whitford, 1989). In general, however the fasting plasma concentration of healthy adults is roughly equal to that in the drinking water, provided that the plasma concentration is expressed as μ mol/L and the water concentration as mg/L or ppm (Guy *et al.*, 1976). Variations are due to individual differences in the rates of removal of fluoride by the kidneys and skeletal tissues. Nevertheless, the general relationship means that plasma fluoride concentrations are not homeostatically regulated, as was once believed. These values are based

largely on studies with young or middle aged healthy adults. They are probably lower in young children and higher in the elderly, but there is paucity of data to support these assumptions. No changes in blood morphology or adverse effects on the haematopoietic system were evident in cows receiving up to 93 ppm NaF for 7.5 years (Shupe *et al.*, 1963).

2.2.5 Fluoride in soft tissues

Short term studies using radioactive fluoride in rats have shown that intracellular fluoride concentrations are 10 to 50 % lower than those of plasma, but they change simultaneously and in proportion to those of plasma (Whitford *et al.*, 1979a). The tissue to plasma ratios of radioactive fluoride are consistent with the hypothesis that hydrogen fluoride (HF) is the form in which fluoride migrates and establishes diffusion equilibrium across cell membranes. Since the pH gradient across the membranes of most cells can be increased or decreased by altering extracellular pH, it is possible to promote the net flux of fluoride into or out of cells. This is the basis for the suggestion that alkalinization of the body fluids is a useful adjunct in the treatment of acute fluoride toxicity (Whitford *et al.*, 1979b, Krishnamachari, 1987). The low retention of fluoride in soft tissues, makes the meat from animals exposed to heavy fluoride loads safe for human consumption.

2.2.6 Fluoride in specialised body fluids

Transcellular fluids are separated from extracelular fluid by an epithelium with peculiar transport properties. Rat, dog, human and horse cerebrospinal fluid, and

cow milk have fluoride concentrations of less than 50 % of plasma. Gingival crevicular fluid fluoride levels are slightly higher (Ca. 10%) than those in plasma, whereas the concentrations in parotid and submandibular ductal saliva are slightly lower. The concentrations in these oral fluids, however change simultaneously and in proportion to those in plasma (Whitford, 1989). The average ratio for the submandibular and parotid secretions are 0.88 and 0.79, respectively. Generally, the fluoride concentration in milk is low in human and animals. Lactogenesis is directly affected by fluoride ingestion (Stoddard *et al.*, 1963, NRC, 1974). In humans, Ekstrand *et al.*, 1981 found that plasma fluoride was poorly transferred to breast milk even in areas where the drinking water had high fluoride levels.

2.2.7 Fluoride metabolism

Hydrogen fluoride (HF) is a weak acid with a pKa of 3.4 and fluoride metabolism is pH dependent. The transmembrane migration of HF occurs in response to acidity of adjacent body fluid compartments. After absorption, fluoride in plasma establishes an equilibrium in calcified tissues , soft tissues, extracellular and intracellular fluids. Some fluoride is lost together with faeces, sweat, milk and urine (Whitford, 1994).

2.2.8 Excretion of fluoride

The major route of fluoride excretion is by the kidney. However, fluoride is also excreted in small amounts by the sweat glands, the lactating breasts, and the gastrointestinal tract. About 90 % of the fluoride filtered by the glomerulus is

reabsorbed in the proximal and distal convoluted tubules. The percentage of the filtered fluoride that is reabsorbed from the renal tubules ranges from 10 % to 90 % (Ekstrand and Whitford, 1988). Ionic fluoride is not plasma protein bound, hence most of the fluoride is excreted through the kidneys. The urinary fluoride excretion reflects the amount of fluoride ingested (Toth and Sugar,1976; Vandeputte, *et al.*, 1977). Excess intake of fluoride leads to increased urinary excretion of fluoride and renal damage may cause high fluoride levels in plasma and bones. Urinary pH is influenced by the composition of the diet, certain drugs (e.g. acetylsalicyclic acid), respiratory and metabolic diseases. A vegetarian diet causes a less positive fluoride balance compared with a meat diet due to the alkaline pH which promotes fluoride excretion. Approximately one half of the fluoride absorbed is excreted rapidly in the urine.

2.3.0 Toxicity of fluoride

Humans, domestic and wild animals, often ingest some fluoride without any adverse effects. Small amounts of fluoride may have beneficial effects such as protective effect against dental caries. On this basis, the element fluorine is considered to be essential (NRC, 1971; Krishnamachari, 1987). On the other hand, the intake of excessive amounts of fluoride can induce either acute or chronic toxicity. A clear distinction must be made between acute toxic effects, which result from a single massive dose, and the chronic toxic effect of large doses spread over a number of years. Chronic fluoride poisoning (fluorosis) is more common than acute fluoride poisoning. A number of adverse effects have been ascribed to

fluorides. Although many claims are unsubstantiated, several have been studied sufficiently to deserve attention, including potential effects on fertility, bone, kidney, thyroid, neurological functions and growth, (Freni, 1994, Zhao and Wu, 1995).

2.3.1 Acute toxicity of fluoride

Acute fluoride poisoning may result from the ingestion of large quantities of soluble fluoride compounds such as sodium fluoride or silicon tetrafluoride. Acute fluoride poisoning usually results from the accidental ingestion of insecticides or rodenticides containing fluoride salts such as sodium fluoroacetate (compound 1080). Initial symptoms are secondary to the local action of fluoride on the mucosa of the gastrointestinal tract and include salivation, nausea, abdominal pain, vomiting and diarrhea. The patient shows signs of increased irritability of the nervous system including hyperactive reflexes, tonic and clonic convulsions (Whitford, 1991). These signs are related to the calcium binding effects of fluoride and may be delayed for several hours. The blood pressure falls, presumably due to central vasomotor depression as well as direct toxic action on cardiac muscle. The respiratory center is first stimulated and later depressed. The lethal dose of sodium fluoride in man is about 5 g however, recovery has been reported in patients ingesting much larger doses, whereas a low dose of 2 g can be fatal. A dose of 5 mg F/kg may be fatal for some children (Whitford and Ekstrand, 1988). The acute effects of inhaling fluorine was observed in experimental animals (Stokinger 1949). When fluorine contacts the skin of animals it produces a thermal type of

burn. Inhalation of fluorine at 300 ppm was fatal to all animals exposed for 3 hours or longer.

In domestic animals, acute poisoning is commonly seen in pigs and is mainly due to accidental ingestion of excess sodium fluoride. Poisoning following the use of insecticide powders (40 % NaF) is uncommon, but it has been described in the dog (Holmes, 1946). The main signs were diarrheoa and fall in milk yield. Volcanic eruptions can cause acute fluorine poisoning in sheep, as the ash may contain upto 2000 ppm of fluoride. The frequency of the symptoms reported in connection with 34 fatal cases of acute fluoride poisoning were described by Roholm (1937).

2.3.2 Chronic toxicity of fluoride

Chronic fluoride toxicosis has been reported in humans, domestic animals and in some wild animals. Chronic fluorosis develops gradually after continuous ingestion of excess fluoride levels in food and water. Chronic toxicity is characterised by dental fluorosis, skeletal fluorosis as well as elevated levels of fluoride in bone, urine and blood. Factors influencing severity of chronic fluoride intoxication include; amount of fluoride ingested, species, age at the time of ingestion, nutritional status, duration of ingestion, solubility of ingested fluoride, health status, stress factors and individual biological responses (Shupe 1972). Malnutrition and protein deficiency intensifies fluorosis.

2.3.3 Mode of action of fluoride in chronic toxicity

The primary effect of fluoride is mainly due to the delaying and alteration of normal mineralisation of the preenamel, predentine, precementum, and preskeletal matrices. Excessive fluoride intake produces dental fluorosis by affecting the teeth during development. Specific ameloblastic and odontoblastic damage is caused by high fluoride intake and varies directly with the levels consumed. Faulty mineralisation results when the matrix laid down by damaged ameloblasts and odontoblasts fails to accept minerals normally. Once a tooth is fully formed, the ameloblasts have lost their constructive ability and the enamel lesions can not be repaired (Whitford and Ekstrand, 1988). Odontoblasts can produce secondary dentine to compensate for fluorotic deficiencies. The dental lesions of chronic fluorosis are accentuated by the rapid wear of the affected cheek teeth, especially if coarse feeds are fed. Oxidation of organic material in the teeth involved result in brown or black discolouration, which is observed in dental fluorosis.

There are two schools of thought concerning the pathogenesis of fluorotic bone lesions. One theory relates high fluoride levels to osteoblastic activity which leads to inadequate matrix and defective, irregular mineralisation. Others researchers suggest that bone lesions are related to the replacement by fluoride ion of hydroxyl radicals in the hydroxyapatite crystal structure of bone substance. This results in a decrease in crystal lattice dimensions. The pathologic results of skeletal fluorosis include, dissociation of normal sequences of osteogenesis, acceleration of bone remodeling, production of abnormal bone (exostosis, sclerosis), and in some cases accelerated resorption (osteoporosis) (Osweiler *et al.*, 1985).

2.3.4 Chronic toxicity of fluoride in humans

In man, the main manifestations of chronic ingestion of excessive amounts of fluoride are osteosclerosis and mottled enamel. Chronic exposure to excess fluoride causes increased osteoblastic activity. Osteosclerosis is a phenomenon whereby the density and calcification of bone are increased. In chronic fluorosis, it is thought to represent the replacement of hydroxyapatite by the denser fluoroapatite. However, the details of the mechanism of its development remains unknown (Ammitzboll *et al.*, 1988). The degree of skeletal involvement varies from changes that are barely detectable radiologically to marked thickening of the cortex of long bones, numerous exostoses scattered throughout the skeleton, and calcification of ligaments, tendons, and muscle attachment to the bone. Severe fluorosis is a disabling disease and is designated crippling fluorosis. Mottled enamel or dental fluorosis is a well recognised entity. The gross changes in mild mottling consist of small, opaque, paper-white areas scattered irregularly over the tooth surface. In severe cases, discrete or confluent, deep brown to black stained pits give the tooth an irregular appearance. Mottled enamel is the result of a partial failure of the enamel-forming cells to elaborate and lay down enamel. Mottled enamel is a non specific response to a variety of stimuli, mainly due to ingestion of excessive amounts of fluoride. The adequate daily dietary intake of macroelements and microelements in man are presented in Tables 2.1 and 2.2.

Table 2.1. Estimated safe and adequate daily dietary (mg/L) intake of selected macroelements in various age groups in man.

Age (yrs)	Sodium	potassium	Chloride
0 - 0.5	115-350	350-925	275-700
0.5-1.0	250-750	425-1275	410-1200
1.0-3.0	325-975	550-1650	500-1500
4.0-6.0	450-1350	775-2325	700-2100
7.0-10.0	600-1800	1000-3000	925-2775
11+	900-2700	1525-4575	1400-4200
Adults	1100-3300	1875-5625	1700-5100

Source: National Academy of Sciences 1990.

Mottling is one of the first visible sign of an excessive intake of fluoride during childhood (Haynes and Murad, 1985). Fluoride ions play a significant role in the prevention of dental caries. Lack of fluoride in the diet may also lead to less solid bone structures and predispose bone to fractures. The border between the beneficial effects of fluoride and the toxic amounts of fluoride is narrow hence more research is needed to establish the optimal intake levels.

Although the main fluoride source is drinking water, the widespread use of fluoride-containing dentifrices may contribute significantly to the total fluoride daily intake. There is no consensus on the fluoride levels acceptable in various

foods and beverages consumed on a daily basis (Rao, 1984). High fluoride levels upto 15 ppm in some commercially marketed fruit juices and carbonated soft beverages in Kenya present a health risk especially to children (Opinya *et al.*, 1990). There is some indication that house hold detergents, pesticides, and fertilizers containing fluoride may be additional sources of fluoride exposure (Oelschlager, 1971; Kitner, 1971). Fluoride content in foods, beverages and water may contribute to fluorosis, (Njenga 1989).

Table 2.2 Estimated safe and adequate daily (ppm) dietary intake of selected microelements in various age groups in man

Age(yrs)	Cu	Mn	F-	Cr	Se	Mb
0-0.5	0.5-0.7	0.5-0.7	0.1-0.5	0.01-0.04	0.01-0.04	0.03-0.06
0.5-1	0.7-1.0	0.7-1.0	0.2-1.0	0.02-0.06	0.02-0.06	0.04-0.08
1-3	1.0-1.5	1.0-1.5	0.5-1.5	0.02-0.08	0.02-0.08	0.05-0.1
4-6	1.5-2.0	1.5-2.0	1.0-2.5	0.03-0.12	0.03-0.12	0.06-0.15
7-10	2.0-2.5	2.0-3.0	1.5-2.5	0.05-0.2	0.05-0.2	0.1-0.3
11+	2.0-3.0	2.5-5.0	1.5-2.5	0.05-0.2	0.05-0.25	0.15-0.5
Adults	2.0-3.0	2.5-5.0	1.5-4.0	0.05-0.02	0.05-0.02	0.15-0.5

Source: National Academy of Sciences 1990.

2.3.5 Chronic toxicity of fluoride in domestic animals

The various sources which contribute to the total fluoride intake in animals have been summarised by the National Research Council (NRC) (1971; 1974), Suttie (1977) and Shupe and Olson (1983). In Norway, aluminium production is the main fluoride emitting industry where gaseous (HF) and particulate fluorides are emitted. Fluoride concentrations in surface waters in Norway are not hazardous to animals (Skjelkvale, 1993).

Excessive fluoride ingestion causes dental and skeletal lesions and in severe cases adversely influence the health and productivity of domestic animals (Shupe *et al.*, 1971; Suttie, 1983; Bunce; 1985 and Krook 1998). Fluoride intake exceeding 5 ppm affects reproduction in cows (Rensburg and Vos, 1966). In Kenya, fluorosis in farm livestock is a problem in volcanic areas especially in animals drinking water from deep boreholes (Said, 1981). This applies mainly to the semi arid zones in the Kenyan Rift Valley. Fluoride intoxication caused loss of body condition and a fall in milk production in a herd of dairy cattle in Machakos, Kenya (Murray, 1967). Commercial feed concentrate and mineral mix with excessive amounts of fluoride caused a drastic decrease in milk production; as much as 1.5 million kg of milk in a dairy herd of 52 to 120 milking cows over a period of 6 years, in U.S.A. (Eckerlin *et al.*, 1986).

In China, long term administration of fluoride at 2.15 ppm in drinking water led to fluorosis in dairy cows in one farm, but not in another. The milk production during the first and the second lactation of the cows from the two farms were not different but milk yield in the third and fourth lactation were lower in cows with

fluorosis (Xiao and Zhu, 1987). This is of some importance especially in zero grazed dairy animals which depend on commercial feed particularly during the dry season, as well as animals reared in the arid zones of Kenya where fluoride levels in water are usually above 2 ppm.

Fluoride tolerance refers to concentrations in the diet which do not cause toxicity under constant exposure. Suttie, *et al.*, (1972) indicated that intermittent exposure to levels in excess of the tolerances may cause increased severity of bone and tooth lesions, even if the annual average is within tolerance limits. The official tolerance levels of fluoride in dairy cattle feed and water are 30 to 40 ppm and 3 to 6 ppm respectively. However, the tolerance values are too high and should be reduced to levels that protect cattle and the farmers, (Eckerlin *et al.*, 1986; Krook 1998). The pathology and symptoms of calves born by fluoride intoxicated cows have been described by Maylin *et al.*, 1986.

Fluoride intoxication in sheep was correlated with volcanic eruptions (Roholm, 1937). In Kenya, growth rate of sheep was significantly reduced (Said *et al.*, 1977) while pregnant ewes fed 30 ppm fluoride had reduced birth weight and reduced wool production (Wheeler and Brock, 1985). Bone meal products with fluoride levels of 1046 ppm dry weight are marketed in Kenya, (Mburu *et al.*, 1989.). Cat food containing fish has been reported to contain high levels of fluoride (Mumma *et al.*, 1986). In the horse, fluorosis is characterised by unthriftness, poor skin and hair coat, dental fluorosis, diffuse hyperostosis and lameness (Shupe and Olson, 1971). Wild ungulates such as antelopes are susceptible to adverse effects of ingestion of excessive amounts of fluoride and primary lesions are found in bones

and teeth, (Shupe *et al.*, 1984). Chronic ingestion of excessive fluoride was associated with agalactiae in commercial fox herds (Eckerlin and Krook, 1986). Captive crocodiles and rats fed on fluoridated water showed deterioration of health, including tumours as reported by Jacobs and Burgstahler (1998).

2.3.6 Fluoride tolerance

Fluoride tolerance has been estimated in different species of animals from feeding experiments and field studies (Table 2.3). The estimates are guidelines because they are associated with several uncertainties, (Suttie, 1980 and 1983; Krook, 1998). Some authors give one tolerance level for "performance" and another lower level for "pathology". The first estimates the amount of fluoride that can be ingested without clinical interferences with normal performance, whereas the second indicates the level at which distinct pathological changes are induced. Animals can have minor dental fluorotic lesions without any impairment of performance. In cattle, dental lesions can be induced at an intake of 20 ppm F in feed, and histopathological alterations in bone are also likely to be detected at this level (Suttie, 1977; 1983). Though the tolerance levels have been estimated, the average daily minimum requirement of fluoride in cattle is unknown.

Table 2.3. Tolerance levels of fluoride in different animal species

Species	feed*(F/ppm)	water**(F /ppm)
Heifers	30 - 40	2.5 - 4
Dairy cows	30 - 40	3.0 - 6
Beef cattle	40 - 50	4.0 - 8
Finishing cattle	100	12 - 15
Sheep	50-60	5 - 8
Lambs	150	12 - 15
Horses	40 - 60	4 - 8
Growing swine	70	5 - 8
Growing dog	50 - 100	3 - 8
Poultry	150 - 400	10 -13
Breeding mink	50	3 - 8

Source: Central Veterinary Laboratory Oslo, Norway, (Turid, 1995).

Key:

*The critical level when feed is the sole source of fluoride. The tolerance is based on sodium fluoride or other fluorides of similar toxicity.
**The tolerance level is dependent on the amounts of water consumed. Hence the lower values should be used as guidelines for active animals in a warm climate.

2.3.7 Toxicity of fluoride in fish and Marine wild life

Fluoride mainly accumulates in skeletal structures of vertebrates and invertebrates. There is limited accumulation of fluoride in soft, edible tissues, with

the exception of fish skin. The process of fluoride deposition in fish is similar to that in higher vertebrates, where the fluoride ions exchange with hydroxyl groups in the hydroxyapatite complex. The displacement of phosphate by fluoride occur in very concentrated fluoride media. In crustaceans, the ultrastructure of the skeleton is more amorphous than in vertebrate, however there is probably a greater proportion of simple Calcium fluoride (CaF) precipitation (Wright and Davison, 1975). Although *Notothenia rossii marmorata*, an antarctic fish, feed mainly on fluoride-containing krill (*Euphausia superba Dana*), crustaceans, polychaets and salps; the tissues of the fish have a low levels of fluoride (Oehlenschlager and Rehbein, 1982)

Arctic char (*Salvinus alpinus*), a fish found in the temperate regions, accumulate fluoride in muscles (16.6 mgF/kg) and bones (1150 ppm) when living in water with 2-20 ppm fluoride (Christensen, 1987). The toxicity of fluoride to fertilised eggs of a fresh water fish, *Catla catla* was demonstrated experimentally in India (Pillai and Mane, 1984). Eggs of rainbow trout (*Salmo gairdneri*) exposed to 1.5 ppm fluoride showed delayed hatching while the lethal concentration (LC_{50}) for the rainbow trout was between 2.7 and 4.7 ppm fluoride in water. However the LC_{50} for carp was between 75 and 91 ppm in water (Neuhold and Sigler, 1960). Fluoride levels of 32 ppm in water could kill oyster populations (Moore *et al.*, 1969). After five days in water of 7 ppm fluoride Perna perna, a mussel showed toxic effects (Hemens and Warwicks 1972).

Krill (*Meganyctiphanes norvegica* and *Euphausia superba*), contains high amounts of fluoride (1300-2400 ppm dry matter); (Soevik and Broekkan, 1979). In

spite of these high levels, feeding of Atlantic salmon and rainbow trout with frozen krill or krill meal, did not markedly increase the fluoride level in the fish flesh (Grave, 1981; Tiews *et al.*, 1982). The fluoride content of the fish bones, however, increased considerably. Although krill contains valuable nutrients, for instance protein and essential fatty acids, the high fluoride content makes krill unsuitable for human consumption (Siebert *et al.*, 1982), but krill might be used in fish feed. High fluoride levels reduce growth rate of oyster spats and render oyster meat unsuitable for human consumption (Nell, 1986).

In several countries, fish and marine organisms contribute significantly to the total daily intake of fluoride (Trautner and Siebert 1985). Fish is an important source of food in Kenya. There are limited publications on fluoride in Kenyan fish (Berg and Haug, 1970, Gikunju, 1990). In Kenya, the effects of high fluoride levels on the growth rate, survival and reproduction of fish is not established. Fish meat contain relatively high concentrations of fluoride than other meats.

2.3.8 Toxicity of fluoride in birds

Birds are less sensitive to fluoride poisoning than other animals and diagnosis of toxicity is more difficult, (NRC, 1974). Hence, emphasis on fluoride toxicity has been on the effects of fluoride on growth rate, feed consumption, bone strength, egg production and egg characteristics. Wild birds of prey have been studied in order to elucidate the effects of fluoride on reproduction.

Increased mortality was reported in laying hens fed 1000 - 1300 ppm F (NaF) (Guenter, 1979). Feeding 800 ppm F to newly hatched turkeys led to 45 %

mortality after eight weeks, whereas reduced growth rate was demonstrated at levels greater than 400 ppm F after 4 weeks (Nahorniak *et al.*,1983). In newly hatched European starling (*Sturnus vulgaris)* an LD_{50} of 17 mg F/kg body weight /day was established while levels of 13 mg F/kg/day caused a reduction in growth, (Fleming *et al.*, 1987). Doses of 500 ppm F (NaF) for six days were lethal to adult American kestrels, (*Falco spaverius*) (Bird and Massari, 1983), and Japanese quail chickens, *Coturnix coturnix japonica*, (Vohra, 1973). In the latter study, doses of 200 ppm F gave reduced growth rate. Suttie *et al.*, (1984) suggested tolerance levels of fluoride in growing poultry of 400 ppm F for Leghorn strain chicken, 300 ppm F for broiler strain chickens, and 200 ppm F for turkey poults. Weber *et al.*, (1969), found reduced growth when one-day-old chickens were fed 500 ppm F (NaF) for four weeks.

Adverse effects of fluoride on reproduction were reported by Pattee *et al.*, (1988). They found a reduction in the number of chicks produced per clutch by Eastern screech-owls, (*Otus asio*) when they were fed fluoride. Short term feeding studies of fluoride to American kestrels did not, however reveal significant effects on clutch size or percentage of eggs hatched (Bird and Massari, 1983, Carriere *et al.*, 1987). In poultry, the effects of fluoride on egg production has been of concern. Feeding 300 or 600 ppm F (NaF) to poultry aged 98 days to 228 days caused a decrease in egg production, especially at the end of the feeding period (Meldi *et al.*, 1983). Other studies have demonstrated a reduction in egg production, as well as decreased feed consumption (Guenter, 1979; van Toledo and Combs, 1984; Hahn and Guenter, 1986).

Eastern screech-owls fed 200 ppm F for 5 to 6 months prior to laying, produced eggs of significantly smaller volume and weight compared with the control group. Egg volume was also reduced in owls fed 40 ppm F (Hoffman *et al.*, 1985). Egg weight was not affected when domestic hens were given similar fluoride doses for 37 weeks (Nogareda *et al.*, 1990). However, Guenter (1979) reported reduced egg size when levels of 1000 and 1300 ppm F (NaF) were fed to laying hens. Feeding experiments using doses less than 200 ppm F did not reveal any decrease in shell thickness of eggs produced by Japanese quails, screech-owls and hens (Smith *et al.*, 1970; Vohra, 1973; Merkley, 1981; Pattee *et al.*, 1988). Bird and Massari (1983) reported a trend towards thicker egg shells when American kestrels were fed 50 ppm F for 10 days. However, the control group had significantly thinner egg shells in a clutch laid before the start of the experiment. In eggs, fluoride is mainly incorporated in the shell, low concentartions are found in the albumen and yolk (Kuhl and Sullivan, 1976; Hahn and Guenter, 1986). Increased bone and egg shell fluoride have been found in birds living near fluoride emitting industries compared to those living in non polluted areas (Henny and Burke, 1990).

Generally, high doses of fluoride induces alterations in egg production and egg characteristics in poultry, however predatory birds and Japanese quail are more sensitive to fluoride exposure. Individual differences are also prominent due to diet, lifespan and disposition (Seel *et al.*,1986). Michel *et al.*, (1984) found that the fluoride disposition rate was higher in egg producing hens (*Gallus domesticus*) than in males and non egg - producing females. Some species have a high tolerance threshold for fluoride probably due to marine adaptation. Excellent

adaptation was demonstrated in Adele penguins (*Pygoscelis adeliae*) feeding on fluoride rich - krill (Culik, 1987). The penguins had extremely high bone fluoride levels without any advers effects. Cloacal discharge has been shown to be the main pathway of fluoride excretion in mallard ducks (*Anas platyrhynchos*) and Adele penguins, indicating that the kidney is the main excretory organ.

In Kenya, domestic and wild birds contribute to the economy of the country as food or as a source of tourist attraction. Unfortunately, little information is available on consequences of high fluoride levels in the diet of the birds, although these birds live in a high fluoride environment.

2.3.9 Fluoride and Cancer

Animal studies are used to predict human risk to chemicals. The chemicals which are carcinogenic in animals are also carcinogenic in humans and vice versa. With the possible exception of arsenic and benzene, all human carcinogens can be modelled in at least one animal species.

The mutagenicity of fluoride supports the conclusion that fluoride is a potential human carcinogen (Caspery *et al.*, 1987). Fluoride acts as a cancer promoter in syrian hamster cells (Jones *et al.*, 1988), furthermore, a two year carcinogenicity study found a significant dose related increase of osteosarcoma in male rats, (Maurer *et al.*, 1990). Jacobs and Burgstahler, (1998) also reported captive rat tumours associated with fluoride in drinking water.

2.4.0 Methods used in fluoride analysis

Methods used for fluoride analysis in biological samples include; direct potentiometric determination, microdiffusion technique, gas-liquid chromatography, high pressure liquid chromatography and colorimetric analysis. Most methods used currently for analysis of fluoride in food utilise the fluoride ion specific electrode for determination of fluoride after the isolation of fluoride (Hemens and Warwicks, 1972, Venkateswarlu, 1975, Dabeka *et al.*,1979, Singer and Ophaug 1979, Duff, 1981 and Spak *et al.*, 1982).

Colorimetric methods over estimated fluoride content due to presence of interfering substances in some of the samples analysed (Belcher *et al.*, 1959; Singer and Ophaug, 1979; Taves, 1983). Proton activation analysis measures total fluoride content in food and the method is not perturbed by the chemical form of fluoride (Shroy *et al.*, 1982). Improved analytical procedures show earlier values for fluoride concentration in human food were too high (Oehlenschlager and Rehbein, 1982.) yet unexpectedly high fluoride values in some food and meat products have been reported (Trautner and Siebert 1985). Open flame ashing followed by diffusion is suitable for fluoride analysis in plants and vegetables, (Shamschula *et al.*, 1979, Njenga, 1989). Thus, development of an accepted method for accurate and precise estimation of fluoride in food and beverages is a high priority for future research (Rao, 1984). Preferably, analysis of foods should be done using a method that determines both free (F-) and bound fluoride. Development of a method for determination of free and bound fluoride in foods is

a prerequisite for determination of the bioavailability of fluoride from various foods (Venkateswarlu 1975; Taves 1983,).

The separation of fluoride from unashed samples by acidic diffusion and determination of the isolated fluoride with some colorimetric methods may give erroneous results (Dabeka *et al.*,1979) as compared to determinations using fluoride ion specific electrode. Birkeland (1970) designed a method which involved fluoride dissolution in a closed double tube chamber. The method was developed specifically for dental tartar samples but it was subsequently modified for analysis of fish tissues (Gikunju, 1990).

2.4.1 Fluoride ion specific electrode

The basic principle of the ion selective electrode methodology is that there is an instantaneous electromotive force of the measuring cell, which is characteristic of the instantaneous concentration or activity of a solution contacting the electrode. When the electrode measures the primary ion the technique is refered to as a direct method. If the material to be determined is allowed to react with other substances before analysis, then the method is termed indirect analytical method (Covington, 1979).

The fluoride electrode consists of a sensing element bonded into an epoxy body. When the sensing element is in contact with a solution containing fluoride ions, an electrode potential develops across the sensing element. This potential, which depends on the level of free fluoride ions in solution, is measured against a constant reference potential with a digital pH /mV meter or specific ion meter

(Covington, 1979). The measured potential corresponding to the level of fluoride ion in solution is described by the following Nernst equation:

E=Eo + S log (A),: E = measured electrode potential, Eo = reference potential (constant), A = fluoride ion activity level in solution and, S = electrode slope (about 57 mV per decade)

The level of fluoride ion, A, is the activity or effective concentration of free fluoride ions in solution. The fluoride ion activity is related to the free fluoride ion concentration (C) by the activity coefficient (a) A = a x C. E is the measurable valuable for each sample.

The main advantage of applying ion-selective electrodes, is the simplicity of the technique, provided there are no other factors which affect the electromotive force established in the measuring cell (Covington, 1979). The electromotive force values are more rapidly stabilized in a stirred solution, especially buffered solutions. The response time for the electrode can change in the course of examination. It is therefore necessary to allow the potential to come to a steady state. The fluoride electrode consists of a sensing element bonded into an epoxy body. When the sensing element (a lanthanum fluoride membrane) is in contact with a solution containing fluoride ions, an electrode potential develops across the sensing element. This potential, which depends on the level of free fluoride ions in solution, is measured against a constant reference potential with a pH/mV meter. The measured potential corresponds to the level of fluoride ions in solution (Orion, 1987).

Ion selective electrode method also offers a wider applicability in fluoride

determination in water, beverages, foods, feeds, biological samples, fertilizers and soils, (Frant and Ross, 1966). The size of the electrode permits analysis of small sample volumes. The following factors are considered before the final solution is analysed: the fluoride ion concentration should be above 10 -6 M (0.02 ppm F), the pH of the water sample should be buffered so as to give maximum fluoride concentration and minimum interference from the hydroxyl ions, the ionic strength should be kept constant and fluoride complexing agents should be inactivated (Orion, 1987).

CHAPTER THREE

FLUORIDE ASSAY METHOD AND DETERMINATION OF FLUORIDE LEVELS IN FRESH SOUP PREPARED FROM MEAT, FISH AND VEGETABLES

3.0 FLUORIDE ANALYSIS

3.1 INTRODUCTION

Fluoride electrode has been used to determine fluoride in drinking water, industrial waste, seawater, air, aerosols, food, urine, beverages and milk, (Jacobsen and Weinstein, 1977). It is easy to use fluoride electrode to measure fluoride in most water samples, but analysis of food, animal feeds and tissue samples require special preparation (Mwaniki and Gikunju, 1995).

The objectives of this study were:

I) to establish the suitability of the method of analysis in water, soup and animal feeds,

II) to compare results from other regional laboratories involved in the analysis of fluoride.

3.2 MATERIALS AND METHODS

3.2.1 Samples

Five hundred grams of animal feed samples were obtained from Kiambu and Thika districts. A total of 114 samples were analysed from 14 different sources and

at three levels of added fluoride namely low, medium and high. Blank tubes (n=30) were used for adding in order to check for any contamination due to cleaning, and handling of laboratory ware and materials directly involved in analysis. Another 84 tubes were used for actual sample addition of fluoride. Low level addition of fluoride was done at 0.10 - 110 μg F, medium addition of fluoride at 120 - 210 μg F and high addition of fluoride at 220 - 310 μg F. The sources were concentrate (n=10), bran (n=10), blank I (n=20), blank II (n=10), mineral salt (n=10), lettuce (n=6), cabbages (n=6), kales (sukuma wiki n=6), spinach (n=6), bovine meat (n=6), broiler meat (n=6), goat meat (n=6), nile perch fillet (n=6) and tilapia fillet (n=6). Recovery percentage for each preparation was determined (Appendix 4).

Borehole water samples of 1 L (n=4) were obtained from four different areas around Nairobi namely: Bulbul, Ongata Rongai, Ndumbuini and Our Lady of Mercy school. Each sample was given a laboratory code label (BB, ORB, DB, and OM) and divided into three equal portions of about 250 ml. Two independent laboratories (Department of Chemistry, University of Nairobi and Kenya Medical Research Institute, KEMRI) analysed each batch of samples while the third batch was analysed in the Department of Public Health, Pharmacology and Toxicology laboratory. From each sample, 250 ml of water remained for future use as reference sample.

3.2.2 Reagents for water and fluid sample analysis

The reagents used for fluoride analysis were: 100 ppm sodium fluoride standard stock solution (Orion, Massachusett, U.S.A.) which was diluted serially to give standards from 0.02, 0.1, 1.0, and 10 mgF/L. Commercial total ionic strength

adjustment buffer III (TISAB III) (Appendix 2), (Orion, Massachusett, U.S.A) buffer; deionised water; plastic beakers pippettes and flasks were used.

3.2.3 Preparation of standard calibration curve

A series of fluoride standard solutions were prepared by serial dilution of the stock solution with volumes of 0.02, 0.1, 1.00 and 10.00 ml of de-ionised water/blank II solution in four different volumetric flasks as described previously (Mwaniki and Gikunju, 1995). The standards obtained were equivalent to 0.02, 0.1, 1.00 and 10.00 mg F/L respectively. A total of 28 calibration curves were then prepared (Appendix 10).

The fluoride concentration in the serial dilutions was determined by immersing the electrode in the mixture and then recording the developed relative electrode potential. Measurements were done starting from the lowest to the highest (0.02, 0.1, 1.0 and 10.0 mg/L). The relative electrode potential measured for each serial dilution was plotted against the fluoride concentration on a four-cycle semilogarithmic graph paper to make the standard calibration curve. From the relative potential measurement for each water or feed sample the corresponding fluoride concentration was determined from the standard curve (Fig.3.1). The detection limit for the method was 0.02 ppm.

3.2.4. Experimental procedure

In preparation of water and other liquid samples for fluoride determination, 0.3 ml of total ionic strength adjustment buffer III (TISAB III) was added to 3 ml of each sample in a 15 ml polypropylene tube as described by Mwaniki and Gikunju, 1995. A fluoride specific electrode was dipped gently into the stirred solution (Covington, 1979). The electrode measures the potential difference created between the reference junction and the solution in contact with the sensitive element. The electrode was connected to an ion analyzer/pH meter which records the potential on a fluorescent screen. In case of standard preparations, 0.3 ml of total ionic strength adjustment buffer III (TISAB III) was added to 3 ml of a known concentration of fluoride in a 15 ml polypropylene tube. A standard curve was then plotted and unknown sample fluoride concentrations were obtained from the standard curve.

3.2.5 Chemical reagents

The following reagents were used for the analysis: perchloric acid (Riedel-de Haen AG, Hannover, Germany), nitric acid (Riedel de Haen AG, Hannover, Germany.), acid mixture: equal parts of perchloric acid and nitric acid, base mixture: sodium hydroxide and trisodium citrate in a ratio of 3: 10; blank II solution was a mixture of sodium hydroxide, tri sodium citrate and acid mixture in a ratio of 15 : 50 : 10, respectively; 100 ppm sodium fluoride was used as stock solution (Orion, Massachusets, USA).

3.2.6 Instruments and Equipment

The following were used: Fluoride combination electrode (96-09 Orion Research Incorporated, Cambridge, Massachuset, USA); Digital pH meter (3020 Orion); Automatic voltage regulator (model CVR 500 AX, Samlex, England); Electrode filling solution (Orion, 90-00-01). pH electrode storage solution (91-00-01, Orion). Magnetic stirrer and a teflon coated bar, 5 mm x 11 mm. Polyethylene tubes (15 ml); Twenty millilitre plastic cups (NDD/TL, Norsk Dental Depot, Oslo, Norway); Pipette tips (1000 µl, 9604, Treff, Degershein, Switzerland). Half- litre Plastic bottle for holding deionised water. Ten millilitre plastic disposable straight pipettes; 500 µl digital transfer pipette (Transferpette, Germany); plastic beakers, measuring beakers, measuring flasks and Metler AE 163, mettler instrumente AG, CH-8606 Greifensee, Switzerland.

De-ionised water was generated from our laboratory for constituting other required solutions. De-ionised water had a neutral pH and a conductivity of zero siemens. Other materials used included: electronic weighing balance, (Metler AE 163, Switzerland), 50 ml glass beakers, 500 ml flat bottomed flask for preparation of tea, electric heating mantle, formalin 10 % for histopathological studies, haematoxylin eosin stain, light microscope, microscope slide.

3.2.7 Determination of fluoride in feed

Samples were analysed using a method reported by Birkeland (1970) and modified by Gikunju *et al.*, 1992. After drying at 105^0C for 24 h, each sample was ground and homogenised. 50 mg of each sample was dissolved in a polypropylene tube containing a mixture of 0.2 ml, 11.6 M perchloric acid and 0.2 ml, 14.3 M nitric acid at 60^0 C for 60 min. The mixture was then buffered to pH 5.2-5.5 with a base mixture of 7.8 M sodium hydroxide and 1.0 M trisodium citrate. The whole dissolution process takes place in a closed double tube arrangement (Birkeland, 1970; Gikunju *et al.*, 1992) to minimise loss of fluoride from the sample. The digested sample was then analysed just like a water sample against the background of a standard. Recovery studies were done in order to determine the suitability of the method.

3.3 RESULTS

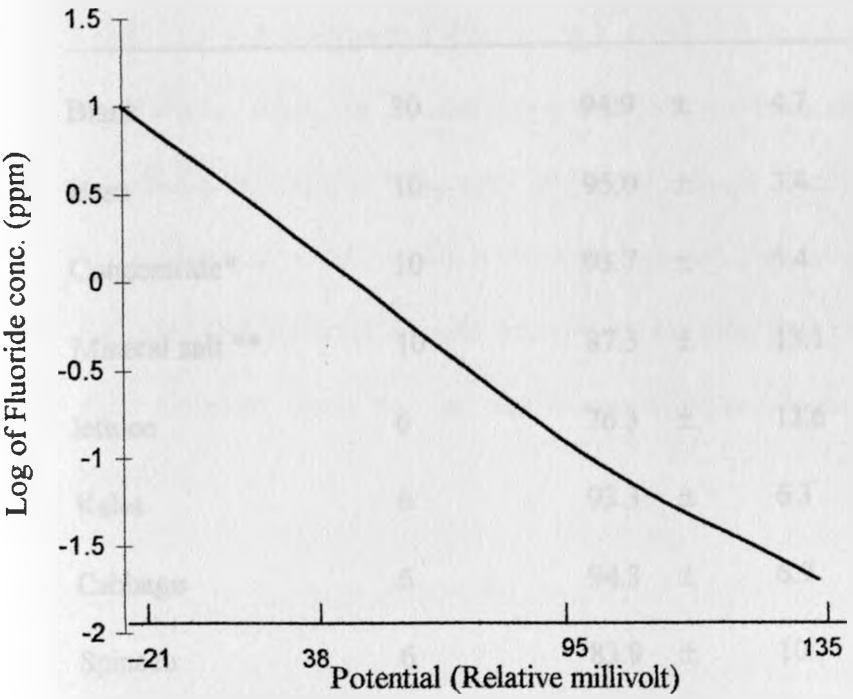
3.3.1 Standard fluoride calibration curves

The Pearson's correlation coefficient (r) was from -0.7495 to -0.9403 while the p-values ranged from 0.000 to 0.0676 for the 28 calibration curves. The mean (± s.d.) r and p values were – 0.8825 ± 0.05 and 0.0136 ± 0.0134 respectively (Table 3.1). A typical standard calibration curve is presented on Fig. 3.1.

Table 3.1 The Pearson's correlation coefficient (r) and the p-values for 28 calibration curves.

Calibration curve No.	(r)	p- value
1.	-0.9085	0.0161
2.	-0.8907	0.0051
3.	-0.8830	0.0034
4.	-0.8862	0.0044
5.	-0.8800	0.0029
6.	-0.8775	0.003
7.	-0.9143	0.0203
8.	-0.9052	0.0088
9.	-0.8653	0.0676
10.	-0.9380	0.0003
11.	-0.9079	0.0145
12.	-0.7788	0.013
13.	-0.8581	0.000
14.	-0.8560	0.0004
15.	-0.8126	0.0064
16.	-0.8859	0.0025
17.	-0.7497	0.0157
18.	-0.9204	0.0128
19.	-0.8998	0.006
20.	-0.9135	0.0125
21.	-0.8998	0.0115
22.	-0.9076	0.0183
23.	-0.9403	0.0369
24.	-0.9095	0.0187
25.	-0.9166	0.0197
26.	-0.7799	0.0203
27.	-0.9112	0.0188
28.	-0.9143	.0205

Fig. 3.1 Fluoride standard calibration curve



3.3.2 Recovery of fluoride from samples

The percent recovery of fluoride from blank I and II was 94.9 ± 4.9 % while the recovery of fluoride from bran, mineral salt and concentrate samples were 95.0 ± 3.4, 87.3 ± 13.1 % and 93.7 ± 5.4 % respectively. The recovery of fluoride from vegetables, meat and fish samples were from 76.3 - 101.5 %. Lettuce had the poorest recovery (76.3 %) while tilapia fillets had a recovery of 101.5 % (Table 3.2)

Table 3.2. Percent recovery of fluoride

Sample	(n)	mean		s.d.
Blank	30	94.9	±	4.7
Bran	10	95.0	±	3.4
Concentrate*	10	93.7	±	5.4
Mineral salt **	10	87.3	±	13.1
lettuce	6	76.3	±	13.6
Kales	6	93.3	±	6.3
Cabbage	6	94.3	±	6.9
Spinach	6	83.9	±	10.7
Bovine meat	6	99.4	±	10.3
Broiler meat	6	95.2	±	10.4
Goat meat	6	77.5	±	4.7
Nile perch fillet	6	100.2	±	5.3
Tilapia fillet	6	101.5	±	5.2

* Dairy meal, ** Afya bora

3.3.3 Interlaboratory comparison of fluoride concentrations in borehole water samples

The mean fluoride levels of borehole water samples bearing code names, BB, ORB, DB and OM were 1.4 ± 0.1, 6.3 ± 0.5, 1.2 ± 1.2 and 5.8 ± 0.6 mg/l respectively, when the results of parallel samples from the three laboratories (Chemistry department, University of Nairobi; Kenya Medical Research Institute and department of Public Health Pharmacology and Toxicology) were considered. Although the individual sample analysis for fluoride from the three laboratories were different, there was no significant difference between the laboratories ($p=0.905$).

3.4 DISCUSSION

3.4.1 Standard Curve

The methodology used to determine fluoride in this study involved procedures which carefully control reagents, standards and buffers and hence minimise contamination and fluoride loss. Fluoride levels in biological, clinical and industrial samples differ due to nature of sample, method adopted and level of contamination (Dabeka *et al.*, 1979, Singer and Opaugh 1979 and Venkateswarlu, 1975). Several sample characteristics may influence the fluoride detected, for instance the presence of excessive amounts of ions which bind fluoride like aluminium and calcium. The concentration of hydroxyl groups and lipids will also influence fluoride detected. Temperature of the sample is also another important factor which can influence the amount of fluoride detected. The above factors form

the basis for modification of the Birkeland's method (1970) which dwelt exclusively on dental tartar samples. Birkeland's method has been modified for analysis of other samples like fish tissues (Gikunju, 1990, Mwaniki and Gikunju 1995).

3.4.2 Recovery percent

The mean recovery of fluoride from blank tubes were: 95.8 ± 3.0 %, 94.4 ± 5.1 % and 95.5 ± 5.2 % for low, medium and high level addition of fluoride respectively. The values indicate that there was minimal contamination of apparatus in the course of fluoride analysis and the cleaning of glassware and plastics was satisfactory.

The mean recovery value of fluoride in vegetables (spinach, kales and lettuce) was 88.0 ± 6.0 %. Gustafsson and Njenga (1988) obtained recovery values ranging from 74 - 103 % while analysing hay, pumpkin leaves, amaranthus spp, lettuce and kales. Recovery of fluoride in meat (broiler, beef and goat), and fish (Tilapia and Nile perch) were 90.1 ± 10.3 % and 100.3 ± 1.0 % respectively. The recovery of fluoride in meat, fish and poultry from this study compares well with recovery values (91-101 %) obtained by Singer *et al.*, (1986). In vegetables, Singer *et al.*, (1986) found recovery values ranging from 59 to 104 %.

Results of the fluoride recovery experiments in bran, concentrates and mineral salts also indicated the reliability of the method. In all the samples the mean recovery was over 75.0 %.

3.4.3 Interlabortory comparison of fluoride analysis

Statistical analysis (ANOVA and t- test) of fluoride results from borehole samples showed that there were significant differences between sample fluoride levels but there was no significant difference between the different laboratories except in case of lab 1 for sample db which had an apparent difference possibly representing an outlier case (Fig.3.2).

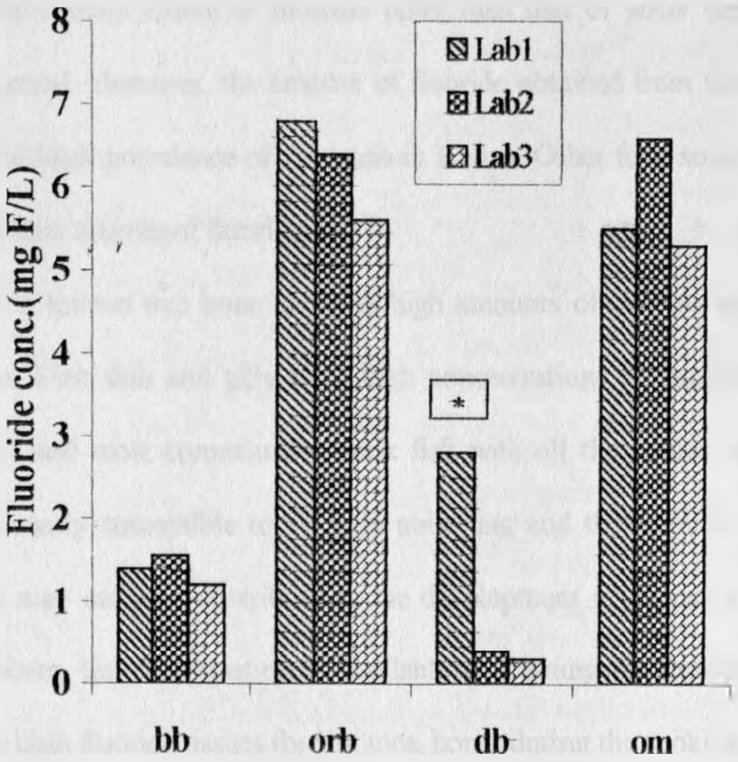
3.4.4 Precision

Reproducibility of results in 10 parallel samples gave a low percent coefficient of variance, indicating that the technique was adequate for analysis in animal feed samples.

3.4.5 Limit of detection and upper range

The limit of determination of fluoride in water, food and feedstuffs was approximately 0.02 ppm with an upper range of 10 ppm. Samples containing fluoride levels above 10 ppm were diluted until the analyte was below 10 ppm and the final results were obtained by multiplying observed values with the dilution factor.

Fig. 3.2 Comparison of four parallel water samples analysed in three different laboratories for fluoride levels



Key: Lab 1 = Kenya Medical Research Institute.

Lab 2 = Department of Chemistry, University of Nairobi.

Lab 3 = Department of Public Health, Pharmacology and Toxicology, University of Nairobi.

* There were apparent differences in sample **db** which were not significant ($p>0.05$).

3.5.0 DETERMINATION OF FLUORIDE LEVELS IN SOUPS PREPARED FROM MEAT, FISH AND VEGETABLES

3.5.1 INTRODUCTION

The dietary intake of fluoride other than that of water depends on the food consumed. However, the amount of fluoride obtained from water cannot account for the high prevalence of fluorosis in Kenya. Other food sources are likely to be important sources of fluoride.

It is known that bone contains high amounts of fluoride as compared to soft tissue. Fish skin and gills have high concentrations of fluoride, (Gikunju *et al.*, 1992) and most communities cook fish with all the tissues intact. Children are particularly susceptible to fluoride poisoning and the amounts provided by such soup may cause or contribute to the development of dental or skeletal fluorosis. However, little information is available concerning the fluoride that leaches out from high fluoride tissues for instance, bones during the cooking process.

In adults, a total daily intake (TDI) of 1.5 to 4.0 mgF is regarded as safe and adequate. In Children, a dosage of 0.06 mg F Kg-1 has been documented as safe, however the TDI values are controversial as more investigation is underway (Cao *et al.*, 1999) The objectives of this study were:

I) to determine fluoride levels in freshly prepared soups, of various foods overcooked for two hours

II) and to relate whether fluoride from soup may be hazardous to the consumer.

3.5.2 MATERIALS AND METHODS

3.5.2.1 Sample collection, preparation and analysis

Meat (beef, broiler and goat), fish (Nile perch and Tilapia) and vegetables (spinach, kales, lettuce and cabbage) were collected from Nairobi markets in amounts ranging from 250 g to 1000 g (Table 3.5.1). The samples were transported to Department of Public Health, Pharmacology and Toxicology laboratory in a cool box and were processed immediately.

About 300 g of fresh sample was cut into small pieces with a scapel blade on a plastic sheet. Vegetables were first washed to remove dust contamination. Each sample was placed in a separate aluminium cooking pot containing 1.5 l of tap water. Ordinary tap water was used as a control. Nine freshly prepared soup samples were obtained. Salt, spices or fat were not added to the sample. The samples were placed on a hot plate and allowed to boil gently. On reaching boiling point, about 3 ml of soup sample was withdrawn at intervals of 15 min. from all the ten samples for 120 min. A total of 90 (3 ml) soup samples were collected. Nine samples were collected from each sample during the period of boiling. The samples were allowed to cool and then analysed for fluoride levels using methods reported previously (Birkeland 1970, and Gikunju *et al.*, 1992), in section 3.2.4

Table 3.5.1. Samples collected from various markets in Nairobi

Sample	quantity	collection area	sample origin
Water (control)	1500 ml	(PHPT*)Lab. tap water	
Cabbage	250 g	Kangemi	Limuru
Bovine meat	500 g	Uthiru	Dagorreti abbattoirs
Broiler meat	500 g	Uchumi, Westlands,	Kenchic
Goat meat	500 g	Uthiru	Maasai land
Lettuce	250 g	City market	Limuru
Spinach	250 g	Kangemi	Limuru
Kales	250 g	Kangemi	Limuru
Nile perch	1000 g	City market	L. Victoria
Tilapia	750 g	City market	L. Victoria

*PHPT= Public Health, Pharmacology and Toxicology

3.5.2.2 Data analysis

Data was entered into dBase IV version 1.1 IBM computer package. The data was then exported (transfered) to Statistix version 3.1 package through Lotus 1 2 3 programme. The statistix computer package was used to provide descriptive statistix. Graphic illustrations were done using Harvad graphics 3.0 and Microsoft Excel programmes.

3.5.3 RESULTS

The mean fluoride concentration in all soup samples was 1.06 ± 1.6 mg /L . The type of food substance used for soup preparation and the time of boiling significantly influenced fluoride levels in the soup. Fluoride levels (ppm) in tilapia, Nile perch, lettuce, spinach, beef, cabbage, kales, goat, and broiler soup were: 5.01 ± 1.27, 2.92 ± 0.54, 0.67 ± 0.34, 0.66 ± 0.28, 0.32 ± 0.05, 0.25 ± 0.05, 0.24 ± 0.03, 0.22 ± 0.04 and 0.16 ± 0.04 respectively (Appendix 5). Soup prepared from tilapia fish had significantly higher fluoride levels than other meat and vegetable soups, ($p < 0.05$). Soup prepared from fish (Tilapia and Nile perch) had fluoride levels significantly higher than in other soup types, while chicken soup had the lowest fluoride levels (Table 3.5.2). The type of soup and duration of boiling significantly influenced ($p<0.05$) the fluoride concentration in the soup. Vegetable soup fluoride levels were not significantly different from one another.

Boiling soup for a long time (2 h) resulted in a more fluoride rich preparation as compared to moderately boiled preparation (30 min.). This was true for fish soup, meat soup, and vegetable soup, (Figs. 3.3, 3.4 and 3.5). Under the same treatment conditions, fish soup had higher fluoride levels than other meat soup preparations (2.5 - 6.4 ppm). Spinach soup had higher fluoride levels among the vegetable preparations (0.42 - 1.3 ppm).

Table 3.5.2 Fluoride levels (mg/L) in freshly prepared soup samples

SOUP TYPE	MEAN		S.D	(n)
Tapwater*	0.1333	±	0.0158	9
Beef	0.3211	±	0.0599	9
Cabbage	0.2544	±	0.0513	9
Broiler	0.1633	±	0.0354	9
Goat	0.2222	±	0.0455	9
Kales	0.2411	±	0.0267	9
Lettuce	0.6700	±	0.3457	9
Nile perch [a]	2.9222	±	0.5472	9
Tilapia [a]	5.0111	±	1.2791	9
Spinach	0.6611	±	0.2844	9
TOTAL	1.0600	±	0.4633	90

[a] significantly higher than the control, Fig. 3.4

* Tapwater control

Fig. 3.3 Fluoride concentration (mg/l) in vegetable soup cooked for 120 min.

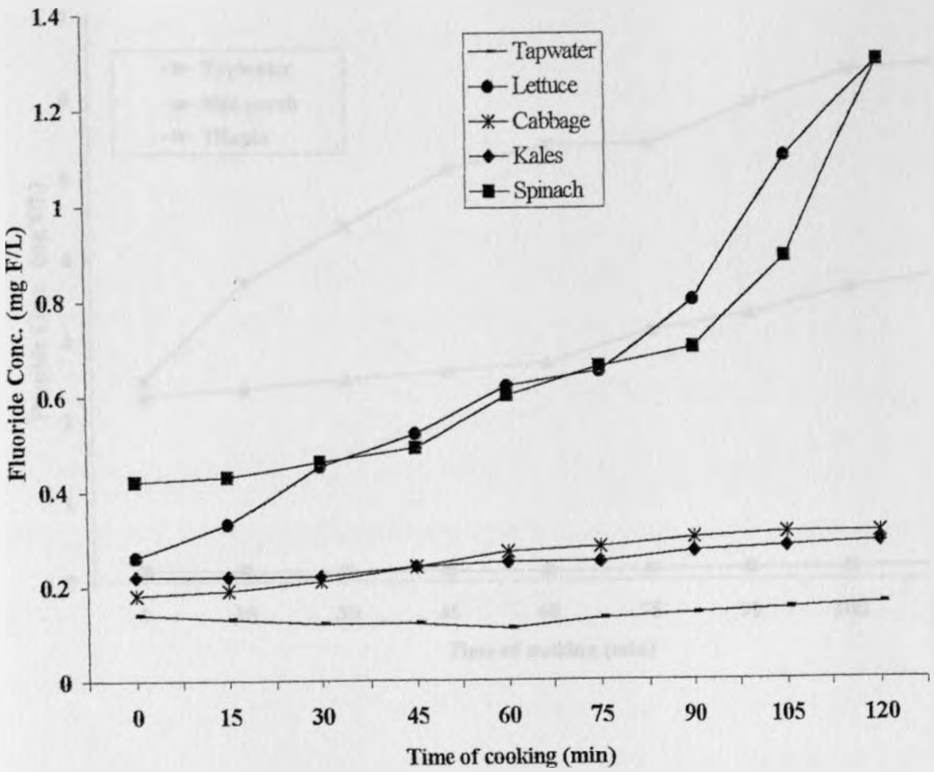


Fig. 3.3 shows that the longer the duration of boiling, the higher the fluoride levels in the soup. The increase in fluoride concentration in the soup depends on the type of vegetable used. Lettuce and spinach soups had higher fluoride levels as compared to kale and cabbage soup (Appendix 5).

Fig. 3.4 Fluoride concentration (mg F/L) in fish soup cooked for 120 min.

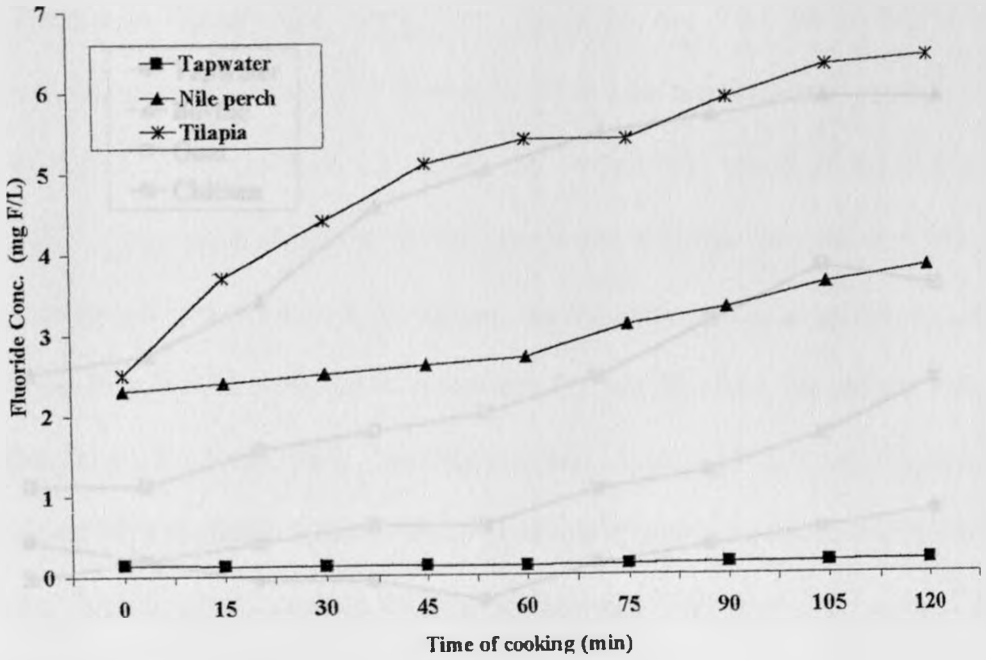


Fig.3.4 shows that the longer the duration of boiling, the higher the fluoride levels in the soup. The increase in fluoride concentration depends on the type of fish used. Tilapia soup had significantly high fluoride levels as compared to Nile perch soup (Appendix 5).

Fig. 3.5 Fluoride concentration (mg F/L) in animal soup cooked for 120 min

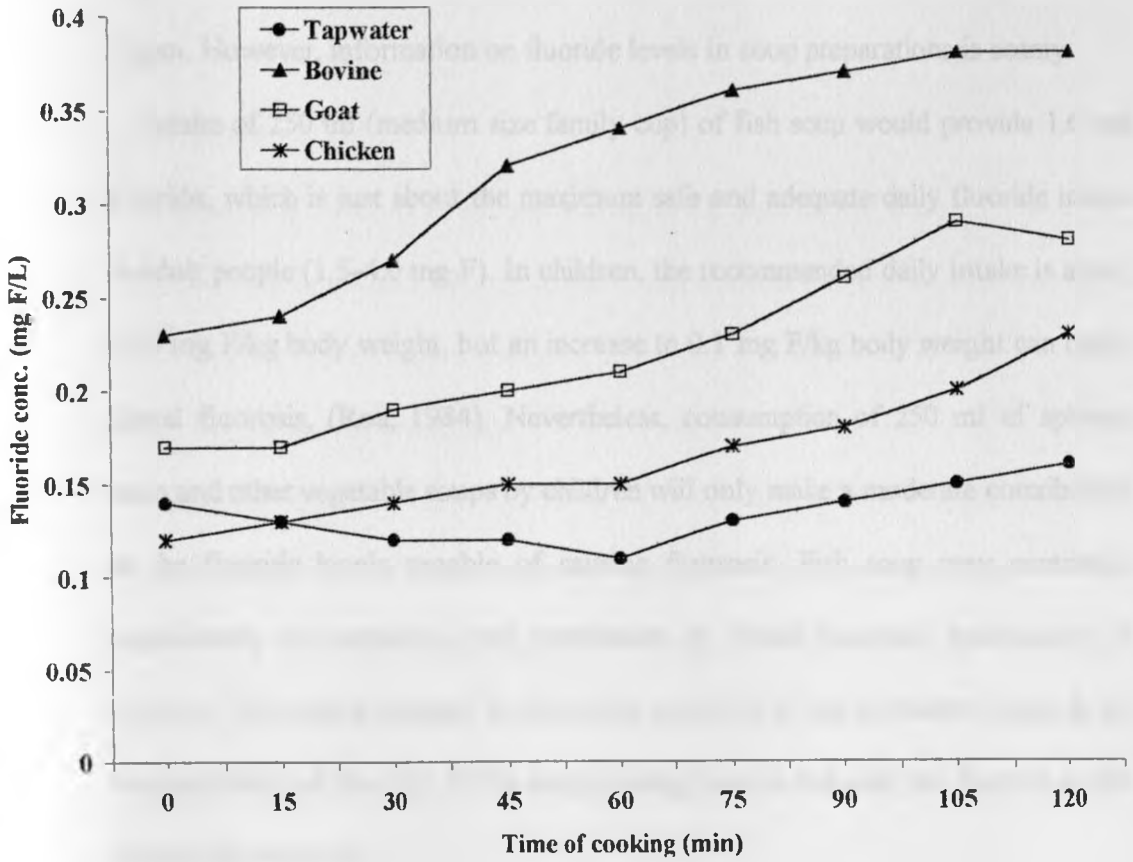


Fig.3.5 shows that the longer the duration of boiling, the higher the fluoride levels in the soup. The increase in fluoride concentration depends on the type of animal meat used. There was no significant difference in fluoride levels (Appendix 5).

3.5.4 DISCUSSION

Gustaffsson and Njenga, (1988) analysed fluoride levels in vegetables and reported that(*Brassica intergrifolia*) and lettuce (*Lactuca sativa*) contained 14.6 - 23.0 and 7.2 ± 3 mg/L respectively. Chen *et al.*, (1998) reported the tolerance limit of fluoride in Chinese rice, wheat flour, vegetables and fresh water fish to be 1.ppm. However, information on fluoride levels in soup preparations is scanty.

Intake of 250 ml (medium size family cup) of fish soup would provide 1.6 mg fluoride, which is just about the maximum safe and adequate daily fluoride intake in adult people (1.5-4.0 mg F). In children, the recommended daily intake is about 0.06 mg F/kg body weight, but an increase to 0.1 mg F/kg body weight can cause dental fluorosis, (Roa, 1984). Nevertheless, consumption of 250 ml of spinach soup and other vegetable soups by children will only make a moderate contribution to the fluoride levels capable of causing fluorosis. Fish soup may contribute significantly to increasing the prevalence of dental fluorosis particularly in children. The data generated in this study could be of use in further research on bioavailability of fluoride. If the soup cooking time is reduced, the fluoride in the soup is also reduced.

CHAPTER FOUR

FLUORIDE LEVELS IN WATER, ANIMAL FEEDS, COW MILK, COW URINE AND MILK PRODUCTION OF DAIRY CATTLE FROM KIAMBU AND THIKA DISTRICTS

4.1 INTRODUCTION

Kiambu and Thika Districts are situated in Central part of Kenya. Most of the available land is suitable for agricultural use. Majority of the farmers are small scale or subsistence farmers involved in a variety of livestock activities such as dairy, pig and poultry production.

Excessive intake of fluoride in water, feed and mineral supplements can adversely affect health, reproduction and production in dairy cattle (Maylin *et al.*, 1986). It is of great importance to the dairy industry to understand the relationship between milk production and fluoride intake in dairy cows. A cross-sectional study was designed and based in Kiambu and Thika districts. The objectives of this study were to:

I) Investigate the levels of fluoride in water, feeds and mineral salts from dairy farms in Kiambu and Thika Districts

II) Determine fluoride levels in urine and milk samples,

III) Determine milk yield (l/day/cow) and to relate milk production to fluoride levels in water and feedstuffs.

4.2 MATERIALS AND METHODS

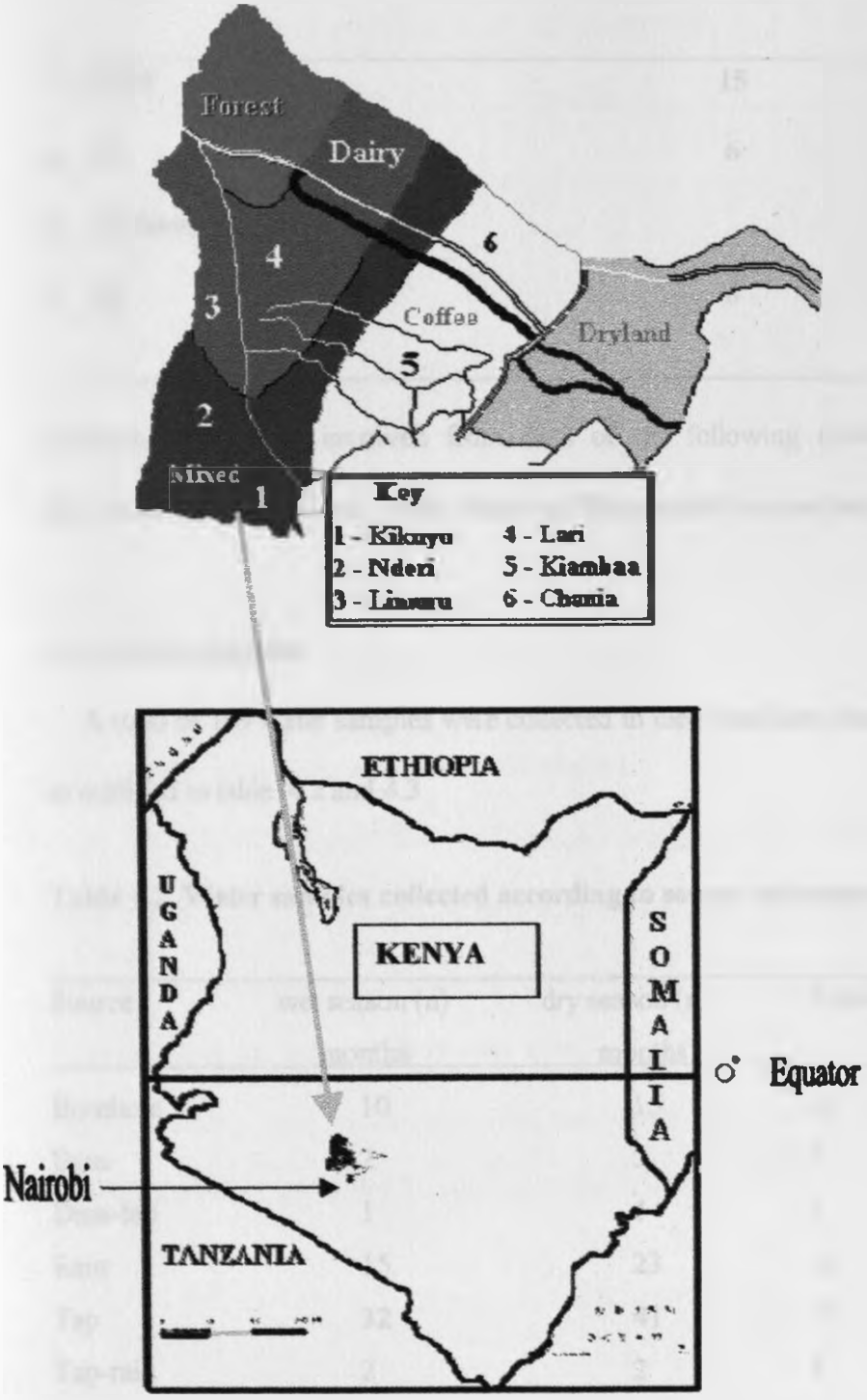
4.2.1 Study area, sampling and data collection

This study involved six dairy societies within Kiambu and Thika districts (Fig.4.1). The dairy societies involved were Chania, Kiambaa, Kikuyu, Lari, Limuru and Nderi. The farms were selected in a two stage stratified random sample technique starting with 17,818 farms belonging to 15 dairy farmers cooperative societies (Table 4.1).

From each co-operative society farmers were randomly chosen for the study. Ten farmers declined to take part in the study leaving 80 farmers for the study with each farmer having dairy animals varying from one to twenty three animals. Animals were identified by tagging or naming in each farm.

Water, dairy feeds, forage, salts and concentrates provided to cattle were collected from each dairy farmer. Milk and urine samples were also collected for fluoride analysis. Samples collected between July and November 1994 were categorised as wet season samples while those collected between January and June 1994 were categorised as dry season samples. A survey questionnaire (Appendix 1) was administered to each farmer.

Fig. 4.1 Sketch map showing the study location



Source: Survey of Kenya map

Table 4.1 Selection of farms in Kiambu and Thika districts

Farms	Dairy co-operative societies
1. 17818	**15**
2. 90	**6**
3. 10 farms withdrew	
4. 80	**6**

Thirteen farms were involved from each of the following societies: Chania, Kiambaa, Lari and Limuru, while Nderi and Kikuyu had fourteen farms each.

4.2.2 Water samples

A total of 149 water samples were collected in clean one litre plastic containers as outlined in table. 4.2 and 4.3

Table 4.2. Water samples collected according to source and season.

Source	wet season (n) months	dry season (n) months	Total
Borehole	10	15	25
Dam	2	5	7
Dam-tap	1	1	2
Rain	15	23	38
Tap	32	41	73
Tap-rain	2	2	4
Total	**62**	**87**	**149**

Table 4.3. Water samples collected according to society and season.

Society	wet season (n) months	dry season (n) months	Total
Chania	7	14	21
Kiambaa	12	18	30
Kikuyu	13	14	27
Lari	9	14	23
Limuru	10	13	23
Nderi	11	14	25
Total	**62**	**87**	**149**

4.2.3 Animal feed samples for fluoride analysis

A total of 104 different feed stuff samples weighing 300 g were collected in clean plastic bags. The number of feed samples collected from Chania, Kiambaa, Kikuyu, Lari, Limuru and Nderi were 18, 24, 22, 5, 16, and 18, respectively. Nineteen different types of feed were found. The most frequently found animal feeds were dairy meal, maize germ, bran and mineral salts. The samples were then transported to the laboratory for analysis (Table 4.5).

Table 4.5 Feed samples collected according to source.

Sample source	(n)	Sample source	(n)
Bran	16	Mineral salts	20
Calf pellet	1	Napier grass (Pennisetum spp)	5
Chicken manure	4	Pyrethrum	1
Comellina bengalensis	1	Saw dust - chicken manure	1
Dairy cubes	1	Star grass	1
Dairy meal	20	Themeda triadra	4
Dog feed *	1	Wheat grains	1
Brewers'waste	2	Wheat bran	3
Magadi salt	1		
Maize germ	18		
Maize stalks	3		
Sub total	**68**		**36**
Total	**104**		

* Not used to feed cattle

4.2.4 Urine samples for fluoride determination

A total of 106 urine samples were collected from the six dairy farmers' co-operative societies. Twenty-one during the dry season and 85 during the wet season. The samples were collected either by manually stimulating the cow or by trapping the urine during normal micturition. About 250 ml of urine were obtained from each cow in a clean 500 ml plastic sample bottle. The samples were placed in

a cool box after labelling each sample with date, farm, owner of farm and cows name/tag. Samples were transported to the laboratory and stored in the freezer at -15^{0}C for fluoride analysis the following day, (Table 4.7).

Table 4.7. Number of urine samples collected according to society and season

Society	wet season (n) (months)	dry season (n) (months)	Total
Chania	0	14	14
Kiambaa	6	16	22
Kikuyu	2	14	16
Lari	5	14	19
Limuru	5	13	18
Nderi	3	14	17
Total	**21**	**85**	**106**

4.2.5 Milk samples for fluoride analysis

A total of 130 milk samples were collected from the six societies in a cross-sectional study. Forty five samples were collected during the dry season while 85 samples were collected during the wet season. 15 ml of milk was obtained from each cow using plastic test tubes. Milk from the four teat of the same cow were

mixed to make one sample. Samples were kept in a cool box and transported to our laboratory the same day and stored in a freezer at –15 °C. (Table 4 8).

Table 4.8. Number of milk samples collected according to society and season

Society	dry season (n) (months)	wet season (n) (months)	Total
Chania	4	14	18
Kiambaa	12	16	28
Kikuyu	11	14	25
Lari	6	14	20
Limuru	8	13	21
Nderi	4	14	18
Total	**45**	**85**	**130**

4.2.6 Milk production

Milk yield values (litres) were obtained by asking the farmer the amount of milk he/she gets from each individual dairy animal in a day during the farm visit. A total of 12 visits were covered during the dry and wet season. A total of 492 responses were processed for the milk production study (Table 4.9). Samples collected between July and November 1994 were categorised as wet season

samples while those collected between January and June 1994 were categorised as dry season samples

Table 4.9. Number of responses on milk production in dairy cattle during the dry and wet season months

Society	wet (n) months	dry (n) months	Total(n)
Chania	21	21	42
Kiambaa	56	56	112
Kikuyu	55	55	110
Lari	35	35	70
Limuru	36	36	72
Nderi	43	43	86
Total	**246**	**246**	**492**

4.2.7. The relationship between fluoride intake and milk production

The fluoride levels in water and feed samples were compared with milk yield in order to establish any association. The data was entered in dBASE IV (Ashton-Tate Corporation, 20101 Halmilton, Ave. Torrance, CA, U.S.A.) and later transferred to Statistix (Analytical software, St. Paul, MN, 55113, U.S.A.) for correlations, scatter plot and regression analysis (Siegel, 1992).

4.3 RESULTS

4.3.1 Fluoride concentration in water

Mean fluoride concentrations (mg/L) were as follows: rain water 0.17 ± 0.09, tap water, 0.22 ± 0.26, borehole, 0.43 ± 0.46, dam-tap, 0.16 tap- rain 0.14 and dam water , 0.17 ± 0.02 respectively, (Table 4.3.1). The mean fluoride concentrations in water from the six dairy farmers co-operative societies arranged in descending order were as follows (mg F/L): Nderi (0.39 ± 0.41), Kikuyu (0.34 ± 0.38), Limuru (0.19 ± 0.23), Lari (0.17 ± 0.07), Kiambaa (0.14 ± 0.05) and Chania (0.12 ± 0.05), (Table 4.3.2). There was a significant difference between fluoride concentration during the wet season and fluoride concentration during the dry season, ($p<0.05$). Borehole water contained significantly high amounts of fluoride as compared to tap, rain, dam, dam-tap and tap-rain water. Society and source of water (Table 4.3.1) significantly influenced fluoride concentration during the dry season ($p= 0.0444$ and $p = 0.0003$).

Table 4.3.1 Comparison of mean fluoride concentration (mg/L) in water samples between different sources and different seasons separately and in combination.

SOURCE	WET (n)	DRY. (n)	COMBINED (n)
Tap water	0.25 ± 0.48 (32)	0.18 ± 0.08 (41)	0.22 ± 0.26 (32)
Dam-tap	0.10 ± - (1)	0.20 ± -(1)	0.16 ± - (1)
Dam	0.15 ± 0.05 (2)	0.15 ± 0.09 (5)	0.17 ± 0.02 (2)
Rain-tap	0.07 ± 0 (2)	0.20 ± 0 (2)	0.14 ± 0 (2)
Rain	0.18 ± 0.12 (15)	0.16 ± 0.09 (23)	0.17 ± 0.09 (15)
Borehole[a]	0.14 ± 0.14 (10)	0.32 ± 0.11 (15)	0.43 ± 0.46 (10)
Total mean	0.20 ± 0.37 (62)	0.20 ± 0.09 (87)	0.24 ± 0.27 (62)
ANOVA, p-value	0.7287	0.0003	0.2420.

[a]. Borehole water contained significantly high amounts of fluoride as compared to other water samples.

Table 4.3.2 Comparison of mean fluoride concentration (mg/L) in water samples between societies and between seasons separately and in combination.

SOCIETY	WET (n)	DRY (n).	COMBINED (n)
Chania	0.08 ± 0.06 (7)	0.17 ± 0.06 (14)	0.12 ± 0.05 (7)
Kiambaa	0.10 ± 0.07 (12)	0.19 ± 0.07 (18)	0.14 ± 0.05 (12)
Kikuyu	0.40 ± 0.73 (13)	0.29 ± 0.14 (14)	0.34 ± 0.38 (13)
Lari	0.16 ± 0.10 (9)	0.17 ± 0.09 (14)	0.17 ± 0.07 (9)
Limuru	0.13 ± 0.08 (10)	0.23 ± 0.42 (13)	0.19 ± 0.23 (10)
Nderi	0.26 ± 0.18 (11)	0.68 ± 1.11 (14)	0.39 ± 0.41 (11)
Total mean	0.20 ± 0.35 (62)	0.28 ± 0.48 (87)	0.24 ± 0.27 (62)
**ANOV, p-value	0.2330	0.0444	0.1300.

** ANOVA = Analysis of variance

4.3.2 Fluoride concentration in feeds

The mean fluoride concentration in feed from the Dairy Farmers Co-operative Societies arranged in descending order were as follows (mg F kg^{-1}): Lari 203.4 ±243.5 (n=6), Kiambaa 91.9 ± 226.3 (n=24), Limuru 67.6 ± 93.4 (n=15), Chania 55.2 ± 73.7 (n=18), Kikuyu 24.1 ± 28.6 (n=22) and Nderi 19.5 ± 11.3 (n=19). The Mean fluoride concentration in feed stuffs were significantly different between

societies (p= 0.0426), and also significantly different due to type of sample analysed (p=0.0001). In addition, there were variations in fluoride concentration possibly due to individual manufacturer processes and the sources of the raw materials.

Four out of six dairy farmers' co-operative societies had mean feed fluoride concentrations above 30 - 40mg/kg, which is the tolerance level in dairy cows. Magadi salt contained the highest amount of fluoride 1051.5 μgF/g when all the feed samples collected were compared. Mineral salts (176.8 ± 165.7 μg F/g) contained fluoride levels beyond the 40.0 μgF/g tolerance limit for dairy cattle while the other feed stuffs were below the limit. Rhodes grass (*Themeda triadra)* had the lowest fluoride concentration of 8.6 ± 3.05 μgF/g, (Appendix 6).

4.3.3 Fluoride concentration in urine

The mean fluoride levels in urine was 1.28 ± 1.0 mg F/kg, (n=106). The mean fluoride concentrations in urine were: 0.62 ± 0.99 and 1.38 ± 0.99 during the wet and dry season, respectively. Kikuyu dairy co-operative society had the highest urine fluoride concentration during the dry and wet season. The mean fluoride concentration in urine were significantly different between societies (p= 0.0304) during the dry season. Further, there were variations in fluoride concentration between societies possibly due to individual differences (Table 4.3.3).

Table 4.3.3. Comparison between fluoride concentration (mg/l) in urine samples between societies and seasons separately and in combination

SOCIETY	WET (n)	DRY.(n)	COMBINED (n)
Chania	-	1.37 ± 0.77 (14)	-
Kiambaa	0.82 ± 1.15 (6)	1.49 ± 0.51 (16)	1.01 ± 0.67 (6)
Kikuyu	1.93 ± 2.08 (2)	2.02 ± 1.65 (14)	2.15 ± 1.76 (2)
Lari	0.81 ± 0.70 (5)	0.79 ± 0.64 (14)	0.75 ± 0.29 (5)
Limuru	0.62 ± 0.57 (5)	1.01 ± 0.54 (13)	0.88 ± 0.44 (5)
Nderi	0.76 ± 0.85 (3)	1.52 ± 1.29 (14)	1.53 ± 1.41 (3)
TOTAL	0.62 ± 0.99 (21)	1.38 ± 0.99 (85)	1.10 ± 0.81 (21)
ANOVA, p-value	0.6218	0.0304	0.2761.

4.3.4 Fluoride concentration in milk

The mean fluoride levels in milk was 0.066 ± 0.14 mg F/kg, (n=130, Appendix 7). The mean fluoride concentrations in milk were: 0.05 ± 0.04 and 0.1 ± 0.22 during the wet and dry season, respectively. Milk samples from Nderi had the highest fluoride concentration during the wet season while Limuru had the lowest fluoride concentration. Milk samples from Chania had the highest fluoride concentration during the dry season while Kiambaa had the lowest fluoride concentration. Society and water-source did not influence milk fluoride levels significantly ($p>0.05$) (Tables 4.3.4 and 4.3.5)

Table 4.3.4 Comparison of mean fluoride concentration (mg/l) in milk samples between societies and seasons separately and in combination

SOCIETY	WET (n)	DRY.(n)	COMBINED (n)
Chania	0.06 ± 0.09 (14)	0.43 ± 0.78 (4)	0.23 ± 0.39 (4)
Kiambaa	0.04 ± 0.006 (16)	0.04 ± 0.01 (12)	0.04 ± 0.007 (12)
Kikuyu	0.04 ± 0.02 (14)	0.09 ± 0.09 (11)	0.07 ± 0.05 (11)
Lari	0.04 ± 0.02 (14)	0.05 ± 0.02 (6)	0.04 ± 0.01 (6)
Limuru	0.04 ± 0.007 (13)	0.10 ± 0.03 (8)	0.07 ± 0.02 (8)
Nderi	0.07 ± 0.03 (14)	0.07 ± 0.01 (4)	0.07 ± 0.02 (4)
TOTAL	0.05 ± 0.04 (85)	0.10 ± 0.22 (45)	0.07 ± 0.11 (45)
ANOVA, p-value	0.2425	0.1087	0.1283.

Table 4.3.5. Comparison of mean fluoride concentration (ppm) in milk samples between societies and seasons separately and in combination.

SOURCE	WET (n)	DRY.(n)	COMBINED (n)
Tap water	0.04 ± 0.02 (41)	0.06 ± 0.04 (17)	0.05 ± 0.02 (17)
Dam/tap	0.04 ± - (1)	0.04 ± -(1)	0.04 ± - (1)
Dam	0.04 ± 0.01 (4)	0.05 ± 0.007 (2)	0.05 ± 0.007 (2)
Rain/tap	0.04 ± 0 (2)	0.04 ± 0 (2)	0.04 ± 0 (2)
Rain	0.05 ± 0.77 (22)	0.18 ± 0.43 (13)	0.11 ± 0.21 (13)
Borehole	0.05 ± 0.03 (15)	0.10 ± 0.09 (10)	0.08 ± 0.05 (10)
TOTAL	**0.05 ± 0.04 (85)**	**0.10 ± 0.24 (45)**	**0.07 ± 0.12 (45)**
ANOVA, p-value	**0.9579**	**0.8254**	**0.6243**

4.3.5 Milk production in six dairy farmers' co-operative societies.

The mean milk yield (l/cow/day) was 3.13 ± 2.79 (492). The mean milk production during the wet and dry season were 3.30 ± 2.63 (246) and 2.96 ± 2.92 (246) respectively. During the wet and dry seasons, Lari had the highest milk yield. The lowest production of milk was recorded from Nderi and Kikuyu during the wet and dry seasons respectively (Appendix 8). There was no significant difference in milk yield between wet and dry seasons ($p>0.05$). However, there was a significant difference in milk production (Table 4.3.6) when wet and dry season samples were combined ($p<0.05$).

Table 4.3.6 Comparison of mean milk production (l/cow/day) between societies and seasons separately and in combination.

SOCIETY	WET (n)	DRY(n).	COMBINED (n)
Chania	3.00 ±2.53 (21)	3.12 ±3.72 (21)	4.43 ± 2.80 (9)
Kiambaa	3.25 ± 2.76 (56)	3.23 ± 3.40 (56)	5.40 ± 2.68 (26)
Kikuyu	3.61 ± 3.45 (55)	2.47 ± 3.06 (55)	6.02 ± 3.28 (22)
Lari	3.90 ± 1.90 (35)	3.61 ± 2.40 (35)	4.58 ± 1.49 (24)
Limuru	3.42 ± 2.05 (36)	3.06 ± 2.37 (36)	4.04 ± 1.89 (30)
Nderi	2.50 ± 2.22 (43)	2.53 ± 2.38 (43)	4.65 ± 2.32 (28)
TOTAL	**3.30 ± 2.63 (246)**	**2.96 ± 2.92 (246)**	**4.65 ± 2.32 (139)**
ANOVA, p-value	**0.2315**	**0.4378**	**0.0006.**

4.3.6 The relationship between fluoride intake and milk production

The fluoride levels in water and feed samples were compared with milk production in order to establish any association. Water fluoride levels in Nderi were the highest, while the milk production and feedstuff values were the lowest. The highest milk production per cow per day was 6.04 ± 3.23 l from Kikuyu Dairy farmers' co-operative society with 0.34ppm fluoride in water and 24 mg F/Kg in the feedstuffs (Table 4.3.7). About 67 % of Dairy farmers' co-operative societies had mean fluoride levels in feed above 30 - 40mg/kg, which is the tolerance level in dairy cows (Table 4.3.6). Milk production was not significantly

influenced by water or feedstuff fluoride concentrations. The linear regression of milk production model was: - MILK YIELD = 3.7508 – 2.57451(Water F-dry) + 6.46025(waterF-wet) – (5.793 x 104) feedstuff fluoride. p = 0.7733

Table 4.3.7. The relationship between fluoride intake and milk production.

	WATER (mg/L)				
Society	Wet (n)	Dry (n)	Combined (n)	Feeds (mg/kg) (n)	Milk yield (l/cow/day) (n)
Chania	0.12 ± 0.05 (7)	0.17 ± 0.06 (14)	0.08 ± 0.06 (7)	55.18 ± 73.66(18)	4.38 ± 2.74(9)
Kiambaa	0.14 ± 0.05 (12)	0.19 ± 0.07 (18)	0.10 ± 0.07 (12)	91.94 ± 226.25(24)	5.45 ± 2.73(26)
Kikuyu	0.34 ± 0.38 (13)	0.29 ± 0.14 (14)	0.40 ± 0.73 (13)	24.13 ± 28.59(22)	6.04 ± 3.23(22)
Lari	0.17 ± 0.06 (9)	0.17 ± 0.09 (14)	0.16 ± 0.10 (9)	240.84 ± 252.19(5)	4.57 ± 31.50(24)
Limuru	0.20 ± 0.23 (10)	0.23 ± 0.42 (13)	0.13 ± 0.08 (10)	64.37 ± 91.14(16)	4.07 ± 1.77(30)
Nderi	0.39 ± 0.41 (11)	0.68 ± 1.11 (14)	0.26 ± 0.18 (11)	18.04 ± 9.50(18)	3.70 ± 1.91 (28)
TOTAL	**0.24 ± 0.28 (62)**	**0.28 ± 0.50 (87)**	**.20 ± 0.36 (62)**	**60.92 ± 136.39(104)**	**4.67 ± 2.41(139)**

4.4 DISCUSSION

The mean fluoride concentration in water from the six societies was 0.29 ppm. The observed mean fluoride levels in dam, rain, tap and borehole water compare fairly well with reported values from other parts of Kenya (Njenga, 1982, Gitonga and Nair 1982, Gikunju *et al.*, 1992, Gikunju *et al.*, 1995) where borehole fluoride levels were higher than in other water sources. However, tap and bore-hole water fluoride levels from Kiambu district were twice the levels found in Molo probably due to climatic factors (Gikunju *et al.*, 1995). The mean fluoride levels in borehole water during the dry season was about five times the amount found during the wet season perhaps due to percolation and evaporation processes. Tap, rain and dam water had almost similar values during the dry and wet seasons.

Most of the water samples analysed in this study had concentrations below the tolerance levels of 3 - 6 ppm in dairy cattle. However, there are reports of reduced milk production and reproduction in dairy cattle drinking water with fluoride concentration as low as 2.15 and 5 ppm (Rensburg and Vos 1966, Xiao and Zhu 1987 and Krook 1998). The maximum fluoride concentration encountered in water in this study was 3.4 mg/l, from a borehole. Rain, dam and tap water are not hazardous to animals. Similar findings were reported on fluoride concentrations in surface waters in Norway by Skjelkvale (1993). However, borehole water need constant surveillance because it may increase total fluoride ingested and hence adversely influence milk production.

This study revealed that dairy cattle mineral salts from Kiambu and Thika districts contained high levels of fluoride (176 mgF/l), while other concentrate samples contained values below the tolerance level of 30 - 40 mg F/kg. Society and sample types were significant in explaining the variations of fluoride levels in feed samples ($p<0.05$) probably due to different agro- ecological zones and where the ingradients are produced. High levels of fluoride (3000 - 13000 ppm) in mineral supplements have been reported to cause arthritis, debility and loss of production in a dairy herd (Griffith, 1977). Other investigators (Suttie 1983, Eckerlin *et al.*, 1986 and Maylin *et al.*, 1986) reported low milk production in dairy herds as a result of exposure to high levels of fluoride in feeds. This study also indicate that dairy cattle from the two districts are exposed to excessive fluoride levels and therefore it is necessary to regulate and state fluoride levels in commercial feed supplements particularly mineral salts.

Urinary fluoride levels ranged from 0.034 to 3.00 ppm. The fluoride levels in urine were significantly different during the wet and dry season ($p<0.05$). Water sources, society and breed are significant in explaining the variations of fluoride levels in urine from the dairy animals ($p<0.05$). Water sources with high fluoride levels also lead to high urinary fluoride levels. Dairy cattle from Kikuyu cooperative society had significantly different fluoride levels in urine as compared to cattle from Lari, Limuru, Kiambaa, Nderi and Chania ($p<0.05$).

Urine fluoride levels of 3 - 10 ppm in dairy cattle are regarded as normal and without adverse effects (Shupe and Olson, 1985). However, Suttie (1985), reported Holstein cows excreting 12.8 ppm in urine while on almost zero amount of fluoride while animals exposed to 200 ppm fluoride excreted 80.3 ppm in urine. The discrepancy in these reports could have been due to analytical errors. Patra *et al.*, (1998) reported industrial fluorosis in cattle and buffallo around Undaipur, India where urinary fluoride levels were about 30 ppm. The fluoride levels of urine encountered in this study do not suggest presence of fluorosis but possibility of low milk production due to high fluoride exposure cannot be ruled out.

The mean milk fluoride levels were fairly low ranging from 0.035 to 0.18 mg/L (Tables 4.3.4. and 4.3.5) and do not depend on seasons, breed of cattle or society ($p>0.05$). According to Shupe and Olson (1985), milk fluoride levels of up to 0.12 ppm are considered normal and cause no adverse effects to dairy cattle. However, levels above 0.15 ppm may cause mild to severe chronic fluorosis. Only Chania cooperative society had milk fluoride level (0.23 ppm) above 0.15 ppm. One milk

sample from Chania had fluoride levels of 1.6 ppm probably due to excess ingestion of fluoride from mineral salts.

The average milk production was 3.13 ± 2.79 (492) l/cow/day while milk production during the dry and wet seasons was 2.96 and 3.29 l/cow/day, respectively. The low production of milk during the dry season was perhaps due to scarcity of feed and water during the dry period. Milk production was independent of breed, society and water source ($p>0.05$). Milk yield of 6.5 litres/cow/day has been reported by Omore *et al.*, (1999) in Kiambu. The difference is probably due to different management practices, level of supplementation and different stages of lactation. Data in this study concur with other findings (Omore *et al.*, (1999) that there is low milk production in Kiambu district dairy cattle, probably due to poor nutrition and excessive fluoride intake.

Exposure to high fluoride levels can cause reduced milk production among other maladies (Suttie 1983, Murray 1967, Eckerlin *et al.*, 1986). Though fluoride levels in most water and feed stuff samples in this study were low, there is some indication that some mineral salts may increase the ingested fluoride substantially to affect milk production adversely (table 4.3.7). The fluoride levels excreted in milk was low, however substantial amount of fluoride was excreted through the urine. In this study milk production was not significantly influenced by water or feedstuff fluoride levels ($p = 0.7733$.)

The fluoride concentration in mineral salts was approximately twice the tolerance level for fluoride intake in cattle. There is a possibility that high fluoride may affect the general health and productivity of the dairy animals. There is need to regulate and state fluoride levels in dairy feed stuff.

CHAPTER FIVE

5.0 TOXIC EFFECTS OF FLUORIDE IN RATS EXPOSED TO DIFFERENT FLUORIDE SOURCES

5.1 INTRODUCTION

Toxicological information on fluoride is obtained mainly from studies conducted in laboratory animals and rat is the species used most frequently. The biological response to excessive fluoride intake varies from skeletal fluorosis to increased bone density (Whitford 1994). Chronic excessive fluoride ingestion decreases the rate of Calcium ion transport across renal tubule endoplasmic reticulum and plasma membranes, and reduces the amount of endoplasmic reticulum and plasma membrane Calcium ion pump protein present in the kidney membranes of rat (Borke and Whitford, 1999). Excessive fluoride exposure may interfere with calcium homeostasis. The objectives of this study were:

I) to identify some of the toxic effects which may occur in rats exposed to graded doses of fluoride in form of sodium fluoride and also from some of the salts known to contain high fluoride levels in Kenya.

II) to obtain data which may be useful in extrapolating fluoride related toxic effects in man and animals.

5.2 MATERIALS AND METHODS

5.2.1 Rats

A total of 100 female wistar weaner rats were obtained from International Livestock Research Centre (ILRI, Nairobi, Kenya) and transported in plastic cages and kept in our laboratory in the animal house at a room temperature of 25 ± 3 ^{0}C, with good ventilation and lighting. Wood shavings were placed in each cage to keep the cage warm and clean. Each group was put in a labelled separate cage with attached feeder trough and graduated watering bottle. The rats were maintained on a diet of mice pencils (Unga Ltd, Nairobi) and tap water *ad libitum* for two weeks before the study started in order to allow for acclimatisation. Fluoride concentration in mice pencils was determined as described in section (3.2.7) and the value taken as background fluoride concentration.

5.2.2 Experimental design

Fresh solutions of fluoride were made daily for each group (5.2.1). Sodium fluoride solutions were made from analytical grade of sodium fluoride powder (BDH,Analar® chemical Co. Ltd, Poole, England.). Rats were randomly divided into 10 groups namely, A (n=10), B (n=10), C (n=10), D (n=10), E (n=10), F (n=10), G (n=10), H (n=10), I (n=10), and J (n=10). Groups A, B, C, D, E and F were fed on 1, 5, 10, 30, 60, 80 mg/L (Or 0.087, 0.42, 0.823, 2.667, 5.45 and 7.804 mg/Kg) sodium fluoride in de-ionised water, respectively. Group G, H, I and J were fed on 2 % Magadi salt solution, deionised water (control), 2 % commercial

mineral salt solution and 2 % tea infusion, respectively. The dose levels were chosen on the basis of the fact that 1 and 5 mg/l represents low fluoride concentration, while 10 and 30 mg/l represents moderate level which may be toxic and 60 and 80 mg/l represents highly toxic levels of fluoride (Whitford, 1991). The oral route of administration was used on voluntary intake.

The feed consumed was weighed and recorded in grams daily for each group. The volume of water (solution) consumed by each group was recorded on a daily basis. Any deaths were recorded immediately. Faecal samples were collected on day 8, 37, 58, 70, 71, 83 97, 299 and 360 in order to assess faecal excretion of fluoride.

Fluoride toxicity was investigated after oral administration from four different sources: sodium fluoride in deionised water, magadi salt in deionised water, commercial cattle salt in deionised water and tea infusion prepared in deionised water.

Body weight of rats from each group was determined for 96 days. From day 96, rats were randomly picked and sacrificed from each group. A total of 58 rats were sacrificed. The organ/bodyweight ratio for each group of rat was assessed. Di-ethyl-ether was used as anaesthesia for the rats, which were sacrificed. After careful dissection of the rat, femur, quadriceps femoris muscle, lower jaw and incisor teeth were isolated and analysed for fluoride levels (3.2.7).

The rats which died were treated in a similar manner to the sacrificed ones. Liver, kidney, heart, and lungs were carefully isolated, weighed and prepared for histopathology according to standard laboratory techniques. A longitudinal and

transverse section of each organ was performed followed by trimming. The tissues were fixed in 10 % formalin followed by dehydration using industrial alcohol. Amylacetate and xylene were used for clearing followed by impregnation and embedding using melted parafin wax (Paraplast plus®, Sherwood medical Co., St. louis, Mo, USA) at 60°C. Blocking was then performed followed by sectioning using a microtome to obtain 4-5 micron sections of tissue. Haematoxylin and eosin stain was used to stain slides for microscopic observation.

5.2.3 Sodium fluoride

An odourless crystalline powder of sodium fluoride, (BDH chemical Ltd, Poole, England) was purchased from House and Mcgeorge Ltd. Nairobi, Kenya. Analytical data were as follows: purity 99.0 %, insoluble matter 0.005 %, acidity (HF) 0.04 %, alkalinity 2.0 ml N %, chloride (CL), fluorosilicate (SiF6) 0.12 %, sulphate (so_4) 0.01 %, copper (Cu) 0.001 %, Iron (Fe) 0.002 %, Lead (Pb) 0.001 % and Potassium (K) 0.01 %.

5.2.4 Magadi salt

A 2-kg of commercial magadi salt for ruminants was obtained from Magadi Soda Company. Ltd, Magadi, Kenya. Apart from being used as a mineral supplement in cattle the salt is also used to improve flavour and to tenderise some traditional foods in Kenya. The salt was used as stock material in preparation of a 2 % solution in deionised water for drinking by experimental rats in group G.

5.2.5 Commercial cattle salt

A 2-kg block of salt (Afya Bora, Unga Ltd, Nairobi) was purchased from a local shop at Uthiru, Kenya and transported to our laboratory. Clean Mortar and pestle were use to grind pieces of the block into fine powder which was then used as stock to make a 2 % solution in deionised water for drinking by experimental rats in group I.

5.2.6 Tea leaves

A 500-g packet of Kenya tea packers (Ketepa Ltd, Kericho, Kenya) was purchased from a local supermarket in Nairobi, Kenya. The tea leaves were used as stock material in preparation of 2 % boiled tea (2 min) infusion for drinking by experimental rats in group J.

5.2.7 Statistical analysis

Data was entered into Dbase IV and latter transferred to Statistix programmes for computation of descriptive statistics, linear models and associations. Analysis of Variance (ANOVA) was performed for group/dose of fluoride comparison during the period of fluoride oral administration. Analyses were done to establish dose-dependent increase or decrease in parameter of interest (body weight, fluoride levels in tissues, faeces and organ weights. Significance among groups was tested with Tukey's Honest Significant Difference (HSD) at a probability level of 0.05. Harvard graphic programme, cricket draw and Microsoft Excel were used for graphic illustrations.

5.3 RESULTS

5.3.1 Clinical observations

There were no deaths observed from groups, A (0.087 mg/kg), B (0.042 mg/kg), C (0.823 mg/Kg), H (control), and J (2 % tea). Ten rats died from groups D, n=1, E, n=2, F, n=2, I n=4, and G n=1. Prior to death, the rats displayed clinical signs such as loss of appetite, rough hair coat, dullness, diarrhoea, unthriftness and death.

5.3.2 Body weight

There was an increase in bodyweight with time in all the groups of rats, ($p<0.05$, Table 5.3.1a and Tables 5.3.1b). Rats receiving fluoride levels upto 10 ppm or 2 % tea had significantly increased bodyweight than the control ($p<0.05$). However, rats receiving 30 ppm, 60 ppm 80 ppm 2 % commercial salt and 2 % magadi salt had significantly low bodyweight (Table 5.3.1b.) as compared to control ($p<0.05$). The control group had the lowest organ/bodyweight ratio while group C had the highest organ/weight ratio (Table 5.3.2.).

5.3.3 Feed and water consumption

Feed and water consumption was between 25-275 g/group/day and 100-280 ml/group/day respectively. ANOVA analysis showed no significant variation with time among and between the groups with respect to the control group. The large variations in feed and water consumption were mainly due to the variations in number of remaining rats.

Table 5.3.1a Mean body weight (g) of rats receiving various fluoride concentrations over a 96-day period

Day	Control*	1 ppm	5 ppm	10 ppm	30 ppm	60 ppm	80 ppm	Magadi**	Commer***	Tea2% infusion
0	183.4±15	183.4±15	189.1±19	191±33	187±21	185±21	195±18	185±21	199±24	201±30
8	173.2±32	185.4±16	193±19	187±29	182±17	185±19	179±16	169±14	174±25	161±23
15	194.6±34	201.2±16	204±17	206±30	198±17	202±20	215±32	181±25	179±27	197±16
22	209.5±33	191.9±15	216±16	220±35	215±17	210±23	221±12	214±19	181±15	217±18
30	223.6±32	217.5±16	200±16	227±34	228±21	220±22	233±12	225±18	216±24	217±28
35	222.9±32	222.0±13	226±15	230±31	225±18	224±29	230±10	227±24	222±27	224±26
44	234.9±32	228.9±18	235±15	237±28	229±20	237±23	241±10	200±22	200±22	244±24
53	257.7±34	256.6±20.5	265±19	267±28	261±21	254±21	260±13	217±29	217±29	266±25
62	265.9±33	263.3±19.9	269±20	272±27	261±21	266±26	267±14	206±25	206±25	270±25
69	269.0±29	266.0±19	273±19	276±29	268±22	271±22	273±15	183±21	183±21	275±26
76	270.6±34	272.0±22	281±20	282±29	268±26	276±24	276±12	182±22	182±22	280±31
85	275.8±31	275.7±20	283±20	301±41	276±30	276±24	279±10	172±18	172±18	281±37
91	-	305.0±55	281±24	314±55	-	-	-	-	-	-
96	279.0±32	270.6±20	279±24	273±26	273±26	269±29	272±10	176±21	176±21	268±23
Mean	274.9±20	264±58	270±63	278±66	242±42	241±42	247±36	197±38	197±38	268±64

Key: * = Deionised water.
**=magadi salt
***= commercial cattle salt
- = missing values (rats not weighed)

Table 5.3.1b Effects of fluoride exposure on mean rat body weight (g) by group/dose.

GROUP	DOSE*	SOURCE	MEAN (G)	(OBS****)	S. D.
H	Control**	Dh20***	261.92	215	55.904
A	0.087	NaF	264.33[a]	205	58.053
B	0.418	NaF	269.97[a]	203	62.798
C	0.823	NaF	277.97[a]	199	66.318
D	2.667	NaF	242.26[b]	141	42.732
E	5.450	NaF	241.54[b]	142	42.327
F	7.804	NaF	246.87[b]	144	36.711
G	2.481	2% msalt#	194.30[b]	139	28.460
I	2.348	2% csalt$	197.24[b]	150	37.768
J	20.847	2% tea	268.37[a]	188	64.179

Overall Analysis of variance (AOVA), p=0.0001

Key

[a] significantly high bodyweight

[b] significantly lower bodyweight

*Dose in mg/kg bodyweight, **Control= baseline fluoride (Contains approximately no fluoride), ***Dh2o = Deionised water, msalt# = magadi salt, csalt$ = commercial cattle salt and obs**** = observations

Table 5.3.2 Effects of fluoride exposure on organ/body weight ratio by group/dose.

GROUP	DOSE*	SOURCE	ORGAN/BODYWEIGHT RATIO			
			Liver	kidney	heart	lung
H	Control**	Dh20***	0.0468	0.0077	0.0511	0.0581
A	0.087	NaF	0.0536	0.0088	0.0593	0.0656
B	0.418	NaF	0.0521	0.0093	0.0586	0.0652
C	0.823	NaF	0.0562	0.0101	0.0622	0.0699
D	2.667	NaF	0.0519	0.0089	0.0580	0.0647
E	5.450	NaF	0.0533	0.0096	0.0586	0.0659
F	7.804	NaF	0.0535	0.0095	0.0596	0.0665
G	2.481	2% msalt#	0.0482	0.0104	0.0531	0.0607
I	2.348	2% csalt$	0.0498	0.0108	0.0548	0.0616
J	20.847	2% tea	0.0484	0.0089	0.0539	0.0602
Mean organ/bodyweight ratio			0.0514	0.0094	0.0569	0.0638

5.3.4 Effects of fluoride exposure on organ weight

5.3.4.1 Liver weight

Liver weight increased as the fluoride exposure was increased upto 10ppm and decreased for higher fluoride exposure. Liver weights of rats were significantly influenced by the dose of fluoride consumed by the experimental rats ($p<0.05$). However, the liver weights of the control rats was not significantly different from

the liver weights of rats in groups D (30 ppm or 2.667 mg/Kg), E (60 ppm or 5.45 mg/Kg), F (80 ppm or 7.804 mg/Kg), and J (2 % tea) ($p>0.05$). Liver weights of rats from group A (1 ppm or 0.087 mg/Kg), B (5 ppm or 0.42 mg/Kg), and C (10 ppm or 0.823 mg/Kg) were significantly higher than those of the control, while liver weight of rats from group G (2 % magadi salt) and I (2 % commercial salt) were significantly lower (Table 5.3.4.1) than the control ($p<0.05$).

5.3.4.2 Lung weight

Lung weight increased as the fluoride exposure was increased up to 10 ppm and decreased for higher fluoride exposure. The lung weight of wistar rats were significantly influenced by the dose of fluoride consumed by the experimental rats ($p<0.05$). However, the lung weight of the control group was not significantly different from the lung weight of rats in groups D (30 ppm or 2.667 mg/kg), E (60 ppm or 5.45 mg/Kg), F (80 ppm or 7.804 mg/kg), and J (2 % tea). Lung weight of rats from group A (1 ppm or 0.087 mg/kg), B (5 ppm or 0.42 mg/kg), and C (10 ppm or 0.823 mg/kg) were significantly higher as compared to the control while lung weight of rats from group G (2 % magadi salt) and I (2 % commercial salt) were significantly lower (Table 5.3.4.2) than the control ($p<0.05$).

Table 5.3.4.1. Mean liver weight (g) of wistar rats after exposure to different oral dosage levels of fluoride.

GROUP	SOURCE	Mg/KG	MEAN (G)	(OBS**)	S. D.
H(control)	Dh2o*	0	12.245	2	2.3264
A	NaF	0.087	14.162[a]	5	2.9137
B	NaF	0.418	14.056[a]	5	1.4798
C	NaF	0.823	15.618[a]	5	2.1439
D	NaF	2.667	12.583[c]	3	2.2477
E	NaF	5.45	12.878[c]	6	1.6335
F	NaF	7.804	13.210[c]	6	1.8813
G	2 % magadi salt	-	9.3740[b]	5	1.0846
I	2 % cattle salt	-	9.8240[b]	5	3.1978
J	2 % tea	-	13.007	3	1.5222

Overall Analysis of variance (ANOVA), p=0.0014

Key

[a] significantly higher liver weight

[b] significantly lower liver weight

[c] no significant difference between the liver weights of control

Dose *** = mg/kg bodyweight OBS** = observations

Dh2o* = De-ionsed water

Table 5.3.4.2. Mean lung weight (g) of wistar rats after exposure to different oral dosage level of fluoride.

GROUP	SOURCE	Mg/KG	MEAN (G)	(OBS**)	S. D.
H(control)	Dh2o*	0	15.21	2	2.7577
A	NaF	0.087	17.335 [a]	5	3.2925
B	NaF	0.418	17.610 [a]	5	1.6904
C	NaF	0.823	19.404 [a]	5	3.0203
D	NaF	2.667	15.667 [c]	3	2.1412
E	NaF	5.45	15.933 [c]	6	1.7820
F	NaF	7.804	16.435 [c]	6	1.9472
G	2 % magadi salt	-	11.798 [b]	5	1.0559
I	2 % cattle salt	-	12.148 [b]	5	3.5597
J	2 % tea	-	16.167 [c]	3	1.5890

Overall Analysis of variance (ANOVA), p=0.0004

Key

[a] significantly higher lung weight

[b] significantly lower lung weight

[c] no significant difference between the lung weights of control

Dose *** = mg/kg bodyweight

OBS** = observations

Dh2o* = De-ionsed water

5.3.4.3 Kidney weight

Kidney weight increased as the fluoride exposure was increased upto 10ppm and decreased for higher fluoride exposure. The kidney weights of wistar rats were significantly influenced by the dose of fluoride consumed by the experimental rats ($p<0.05$). The kidney weight of the control group was not significantly different from the kidney weights of rats in groups G (2 % Magadi salt). The kidney weight of rats from groups A (1 ppm or 0.087 mg/kg), D (30 ppm or 2.667 mg/kg), E (60 ppm or 5.45 mg/kg), F (80 ppm or 7.804 mg/kg), and I (2 % commercial salt) were not significantly different from one another. Kidney weights of groups J (2 % tea), B (5 ppm or 0.42 mg/kg), and C (10 ppm or 0.823 mg/kg) were significantly increased (Table 5.3.4.3) as compared to the control ($p <0.05$).

Table 5.3.4.3. Mean kidney weight (g) of wistar rats after exposure to different oral dosage level of fluoride.

GROUP	SOURCE	Mg/KG	MEAN (G)	(OBS**)	S. D.
H(control)*Dh2o*		0	2.0100	2	0.1556
A	NaF	0.087	2.3280[b]	5	0.2774
B	NaF	0.418	2.5100[a]	5	0.0941
C	NaF	0.823	2.8240[a]	5	0.4850
D	NaF	2.667	2.1767[b]	3	0.1973
E	NaF	5.45	2.3267[b]	6	0.1812
F	NaF	7.804	2.3517[b]	6	0.3667
G	2 % magadi salt	-	2.0260[c]	5	0.1937
I	2 % cattle salt	-	2.1460[b]	5	0.3932
J	2 % tea	-	2.4100[a]	3	0.1153

Overall Analysis of variance (ANOVA), p=0.0097

Key

[a] significantly higher kidney weight

[b] not significantly different

[c] no significant difference between the kidney weights of control

OBS** = observations

Dh2o* = De-ionsed water

5.3.4.4 Heart weight

The heart weight of wistar rats were significantly influenced by the dose of fluoride consumed by the experimental rats ($p<0.05$). The heart weight of the control group was not significantly different from the heart weight of rats in groups D(30 ppm or 2.667 mg/kg), E (60 ppm or 5.45 mg/kg), F (80 ppm or 7.804 mg/kg), and J (2 % tea). The heart weight of rats from groups A (1 ppm or 0.087 mg/kg), B (5 ppm or 0.42 mg/kg), and C (10 ppm or 0.823 mg/kg) were significantly higher than the control while heart weight of rats from groups G (2 % magadi salt) and I (2 % commercial salt) were significantly decreased than the control (Table 5.3.4.4).

Table 5.3.4.4. Mean heart weight (g) of wistar rats after exposure to different oral dosage level of fluoride.

GROUP	SOURCE	Mg/KG	MEAN (G)	(OBS**)	S. D.
H(control)	*Dh2o	0	13.390	2	2.4749
A	NaF	0.087	15.6740[a]	5	3.2192
B	NaF	0.418	15.810[a]	5	1.1735
C	NaF	0.823	17.300[a]	5	2.3068
D	NaF	2.667	14.047[c]	3	2.1584
E	NaF	5.45	14.167[c]	6	1.8464
F	NaF	7.804	14.703[c]	6	1.9342
G	2 % magadi salt	-	10.320[b]	5	0.9871
I	2 % cattle salt	-	10.808[b]	5	3.3534
J	2 % tea	-	14.470[c]	3	1.5006

Overall Analysis of variance (ANOVA), p=0.0006

Key

[a] significantly higher heart weight

[b] significantly lower heart weight

[c] no significance different between heart weights of control

OBS** = observations

Dh2o* = De-ionsed water

5.3.5 Fluoride concentration in rat tissues

The dose of fluoride administered, duration and the tissue type analysed significantly influenced fluoride concentration ($p<0.05$). Fluoride concentration in tissues (muscle, femur incisor and jaw) of the control group was not significantly different from the fluoride concentration of rats in groups A (0.087 mg/kg), B (0.042 mg/kg), C (0.823 mg/kg), I (2 % csalt), G (2 % msalt) and J (2 % tea). The fluoride concentration in groups, D (30 ppm or 2.667 mg/kg), E (60 ppm or 5.45 mg/kg) and F (80 were significantly higher as compared to the control, (Table 5.3.5.1). The muscles contained the lowest fluoride levels while the lower jaw-bone contained the highest fluoride levels. There was no significant difference between fluoride levels in femur and incisor bones ($p>0.05$). Fluoride concentration from the lower jaw-bone was significantly higher than fluoride in muscles and other tissues (Table 5.3.5.2).

Table 5.3.5.1 Mean fluoride concentrations (mg/kg) in tissues

GROUP	SOURCE	Mg/KG	MEAN(G)	(OBS*)	S. D.
H(control)**Dh2o		0	326.2 [c]	13	215.3
A	NaF	0.087	291.6 [c]	20	213.6
B	NaF	0.418	330.2 [c]	17	214.1
C	NaF	0.823	363.6 [c]	17	246.0
D	NaF	2.667	612.6 [a]	27	560.3
E	NaF	5.45	1056.7 [a]	28	916.9
F	NaF	7.804	1622.8 [a]	31	856.4
G	2 % magadi salt	-	569.0 [c]	13	215.3
I	2 % cattle salt	-	460.0 [c]	42	445.6
J	2 % tea	-	285.9 [c]	14	142.5

Overall Analysis of variance (ANOVA), p=0.0001

Key

[a] significantly higher fluoride levels

[c] not significantly different from the fluoride levels of control

*Dh2o = Deionised water

OBS* = observations

Table 5.3.5.2 Mean fluoride levels in wistar rats by tissue type.

TISSUE	MEAN (mg/kg)	(OBS*)	S.D.
Muscle	19.1	50	28.0
Incisor teeth	694.0	67	536.4
Femur	730.5	60	576.8
lower jaw	1063.5[a]	65	829.6

Overall Analysis of variance (ANOVA), p=0.0001

Key

OBS* = observations

[a] significantly higher fluoride levels

5.3.6 Fluoride levels in faecal samples

A total of 148 observations were made on fluoride excretion in faeces. The mean fluoride level was 26.98 ± 33.3 mg/kg of faeces, with a range of 0.14 - 158.2 mg/kg. Faecal excretion of fluoride was significantly influenced by time (Table 5.3.6.2) and not by the dose (Table 5.3.6.1).

Table. 5.3.6.1 Mean faecal (mg/kg) fluoride concentration according to dose

DOSE (mg/kg)	MEAN	(OBS*)	S. D.
0 [a]	20.348	56	24.550
2 [b]	29.133	52	35.164
5	32.161	16	37.576
7	40.117	10	53.446
20	30.154	14	34.067

Overall Analysis of variance (ANOVA), p=0.3448

Key [a] = 0, 0.087, 0.418 and 0.823: [b] = 2.667

OBS* = No. of observations

Table. 5.3.6.2 Faecal excretion of fluoride according to time of study

DAY	MEAN (Mg/KG)	(OBS*)	S.D.
8	34.906	18	36.146
37	9.1756	18	10.234
58	11.188	20	12.536
70	45.016	20	48.444
71	41.846	10	43.346
83	28.171	20	30.330
97	19.693	20	22.993
299	23.475	8	24.759
360	36.519	14	39.118

Overall Analysis of variance (ANOVA), p=0.0059

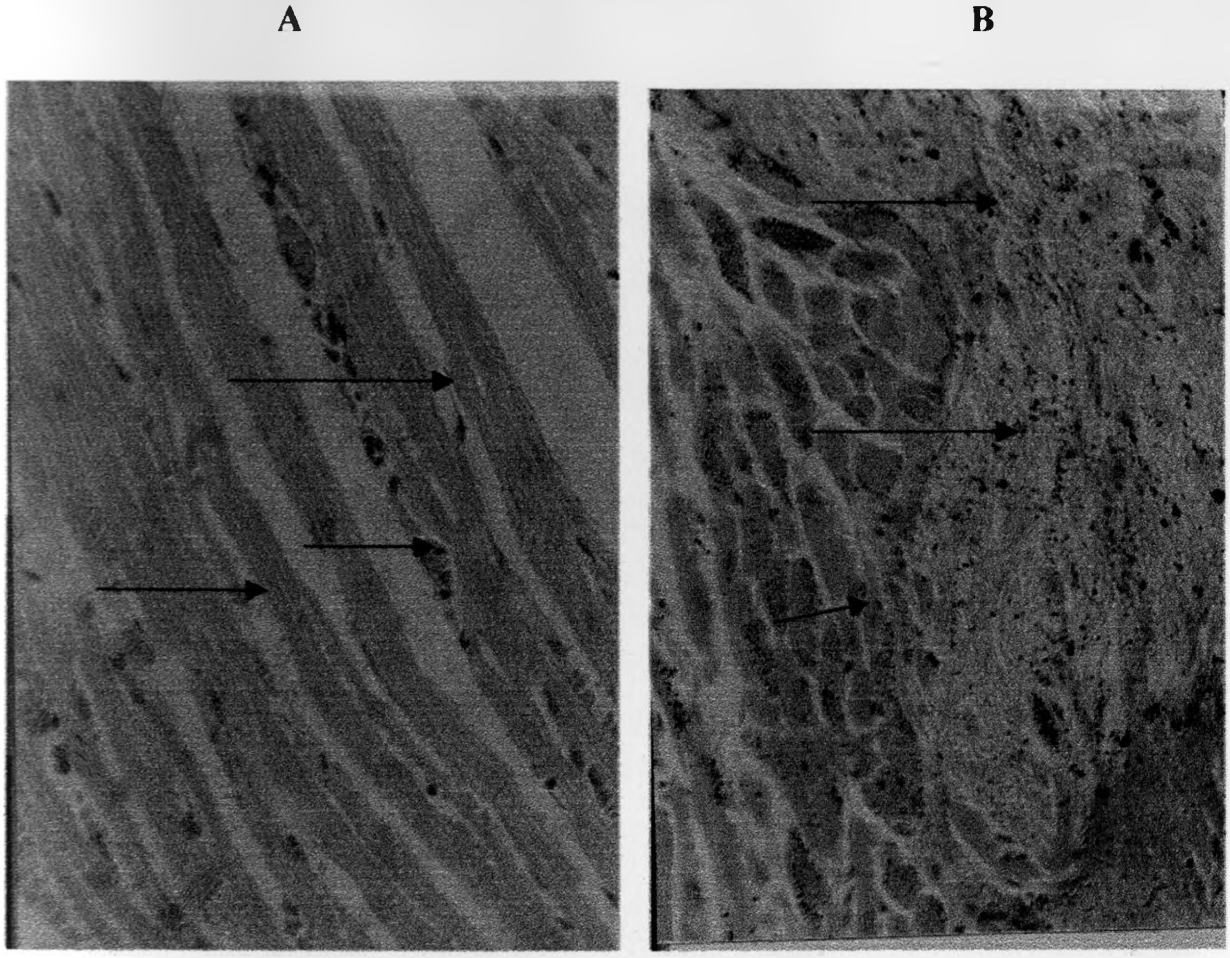
5.3.7 Histopathology of the rat tissues

After an initial screening of the prepared slides, it was observed that fluoride affected the kidney, liver, lung and heart tissues. Increased concentrations of fluoride cause toxicity to soft tissues and may induce pathological changes (Fig.5.3.7.1-4). Rats fed on fluoride at levels above 30 ppm in water had myocardial degenerative changes and coagulative cardiac muscle necrosis. The control rat myocardium indicates normal cell details including intercalated discs (Fig.5.3.7.1)

Rats fed on fluoride at levels above 30 ppm in drinking water displayed complete obliteration of the nephron, hyaline and necrosis of the kidney. The control rat kidney indicates normal glomerulus, tubules and blood vessels (Fig.5.3.7.2). Rats receiving over 30 ppm fluoride in drinking water had liver changes such as fatty degeneration, enlarged sinusoids, atrophic cells and necrosis. The control rat liver indicates normal hepatic cell details including central vein (Fig.5.3.7.3). Rats fed on fluoride at levels above 30 ppm in drinking water displayed large alveolar, thickening of the inter-alveoli septum, fluid and blood in the alveoli spaces of the lung tissues. The control rat lung indicates normal alveoli (Fig.5.3.7.4).

From day 579 of fluoride administration one rat from each of the groups H (control), A, C, J (tea 2 %) had swellings (Appendix 9). The swellings from A , J and C10 were located on the abdominal area close to the inguinal region while those the control were found on the neck region. Microscopic examination revealed the mass from H (conrol) to be a granulomatous lesion while the mass from group A was an adenocarcinoma. The mass from group J was a fibroma while that from C10 was an adenocarcinoma, (Figure 3.5.7.5).

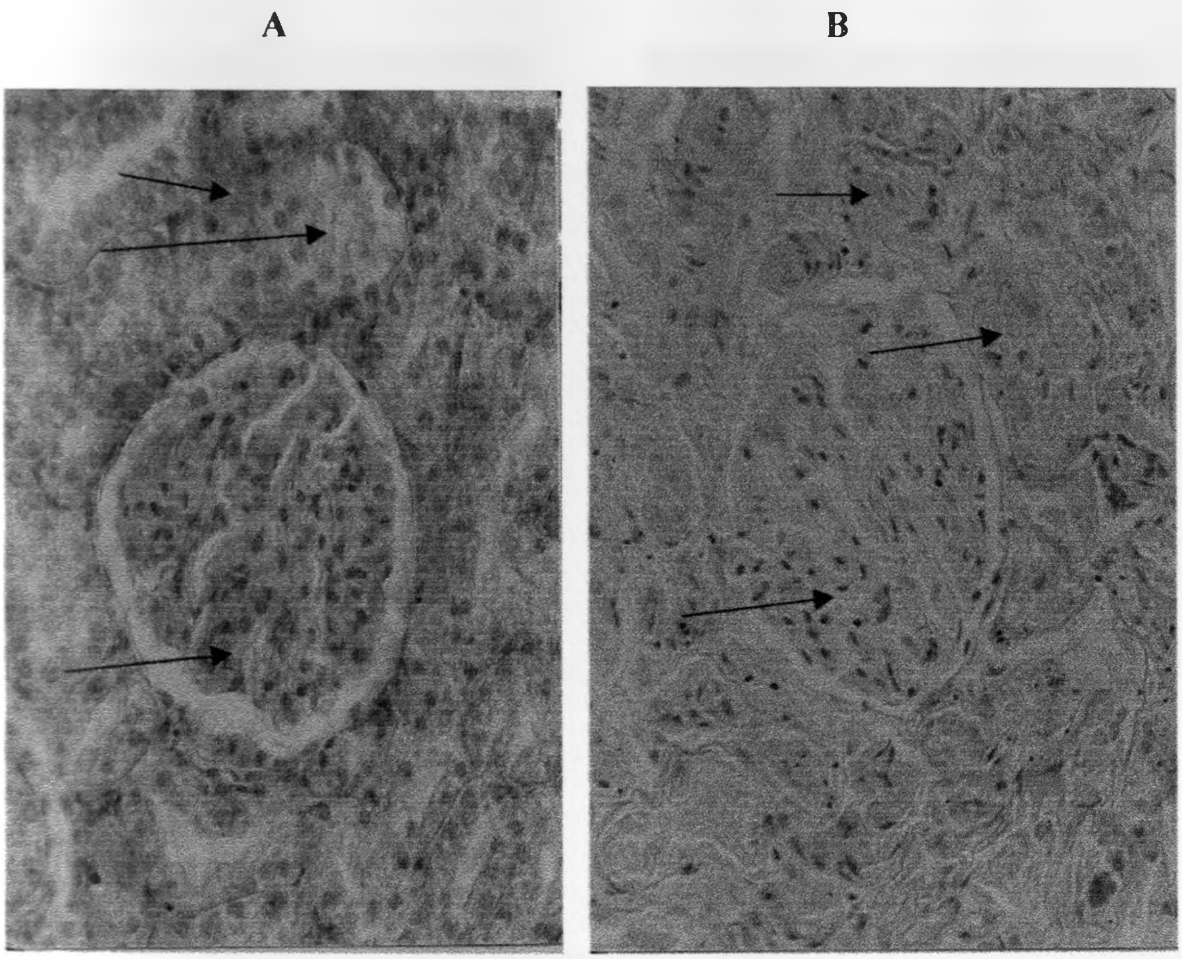
Fig. 5.3.7.1 Sections from the heart of rats:



A, normal heart muscle from a rat given de-ionised water for 165 days, showing nuclei, intercalated discs and branching of muscle fibres.

B, heart from a rat given 80 ppm fluoride for 165 days, showing myocardial atrophy, degeneration and necrosis, haemorrhage and loss of fibre alignment (arrows), H & E stain, x 40.

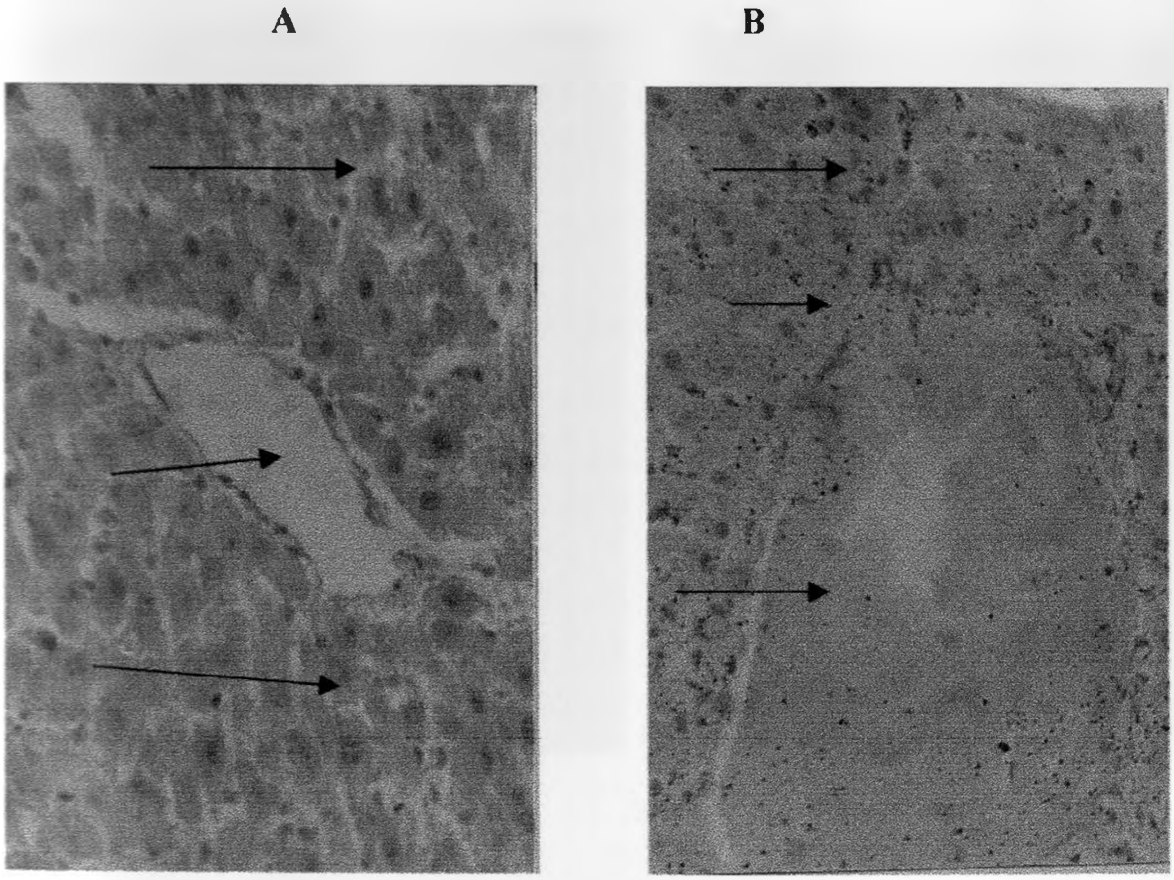
Fig. 5.3.7.2 Sections from the kidney of rats:



A, normal kidney from a rat given de-ionised water for 165 days, showing glomerulus, renal tubules and blood vessels (arrows).

B, kidney from a rat given 80 ppm fluoride for 165 days, showing glomerular hyalination, tubular degeneration and necrosis, (arrows), H & E stain, x 40.

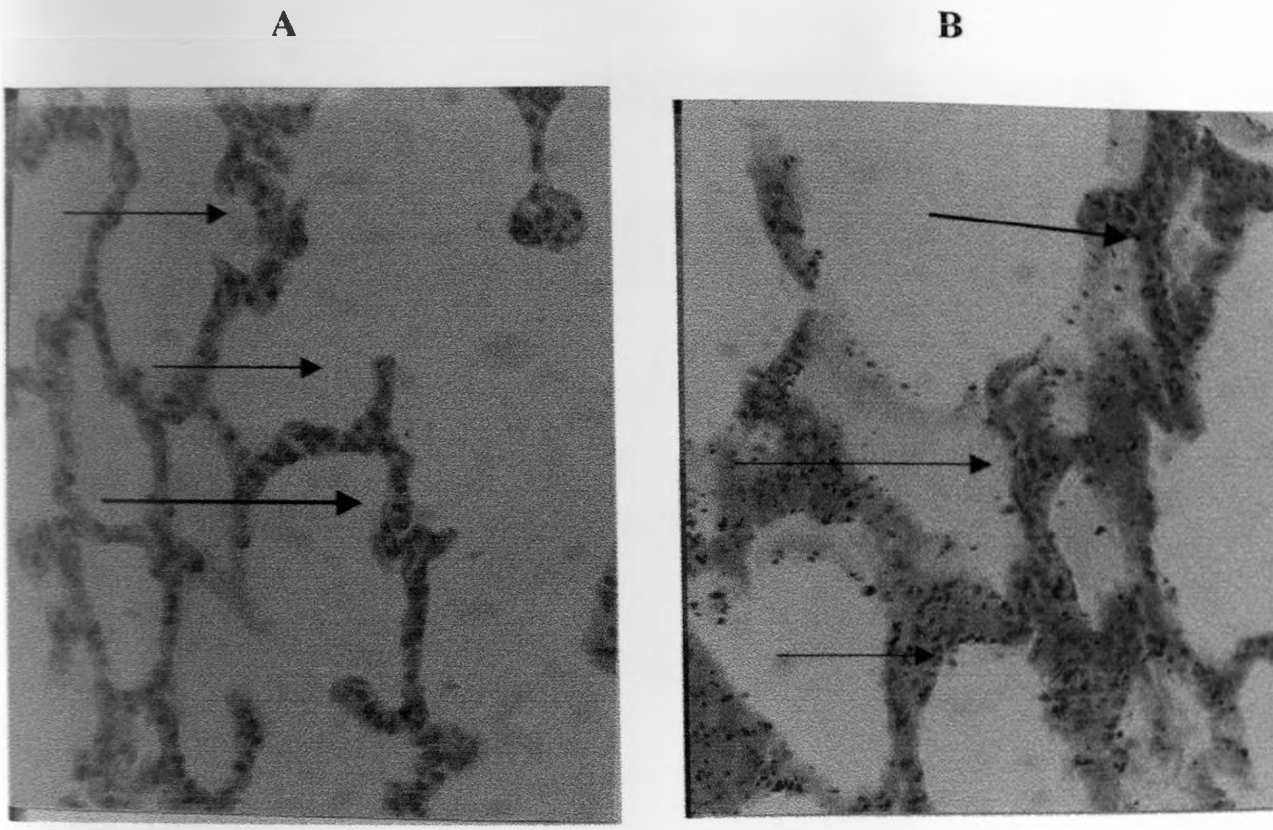
Fig. 5.3.7.3 Sections from the liver of rats:



A, normal liver from a rat given de-ionised water for 165 days, showing hepatic cells and central vein (arrows).

B, liver from a rat given 80 ppm fluoride for 165 days, showing hepatic degeneration, and necrosis characterised by small and large nuclei and cellullar debri, (arrows), H & E stain, x 40.

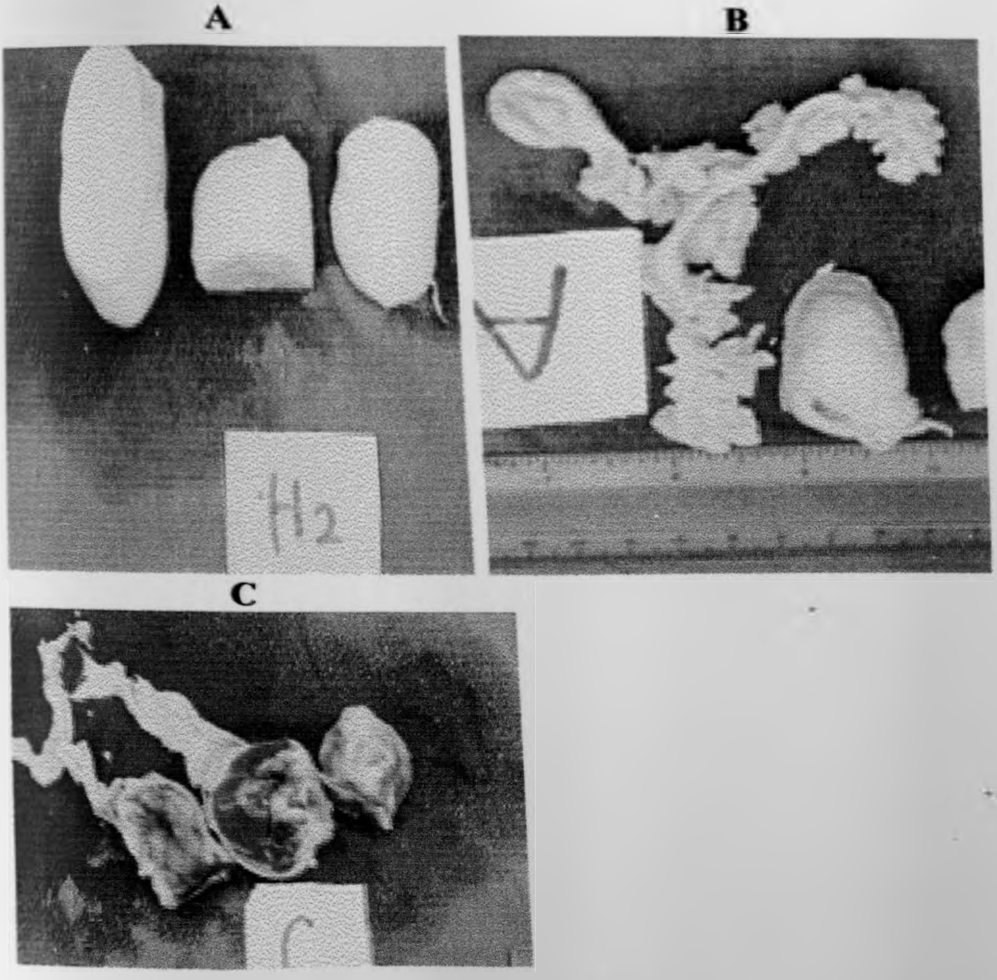
Fig. 5.3.7.4 Sections from the lung of rats:



A, normal lung from a rat given de-ionised water for 165 days, showing alveoli and inter-alveoli septum (arrows).

B, lung from a rat given 80 ppm fluoride for 165 days, showing thickening of inter-alveolar septum , oedema, necrosis and obliteration of alveoli (arrows), H & E stain, x 40.

Fig. 5. 3. 7 .5. Wistar rat tumours



A, Fibroma attached to the pubic vein from one rat among group J, given 2 % tea solution for 646 days,

B, adenocarcinoma of the uterus from one rat among group A, given 1 ppm fluoride for 606 days,

C, adenocarcinoma of the uterus from one rat among group C, given 10 ppm fluoride for 579 days.

5.4. DISCUSSION

The clinical signs observed in rats from group D, E, F, G and I such as loss of appetite, rough hair coat, dullness, diarrhoea, unthriftness and death have been reported previously (Freni, 1994, Zhao and Wu, 1995) in acute fluoride toxicity. The symptoms are secondary to the local action of fluoride on the mucosa of the gastrointestinal tract. Signs of the nervous system such as hyperactive reflexes, tonic and clonic convulsions which are related to calcium binding effects of fluoride were not observed in this study. However, convulsions may be periodic and difficult to observe. The lack of toxicity signs in rats from groups H (control), A (0.087 mg/kg), B (0.042 mg/kg), C (0.823 mg/kg) and J (2 % tea) was perhaps due to low dose levels and the fact that fluoride from tea may be complexed and therefore not available for absorption. However, Cao *et al.*, (1999) and Kavanagh *et al.*, (1998), showed that fluorosis was significantly correlated with tea consumption and further investigation is necessary to determine the significance of tea infusion on dental fluorosis in man.

Overall there was an increase in bodyweights in all groups during the study period ($p<0.05$). The amount of fluoride ingested and the duration of exposure influenced bodyweight and organ-weight significantly, ($p<0.05$). Fluoride levels above 10 ppm caused a significant decrease in body-weight while levels below 10 ppm caiused a significant increase in body-weight ($p<0.05$). Similar findings were also reported by Shupe (1972), but he did not establish the level of optimal performance. Groups H1 (commercial salt 2 % solution) and G1 (Magadi salt 2 % solution) rats demonstrated severe reduction in bodyweight perhaps due to

potentiation of fluoride by other constituents of the supplements. Feed and water intake were not influenced significantly by the dose of fluoride offered. As the dose of fluoride (NaF) increased liver, lung and heart weights also increased as observed in groups A (0.087 mg/kg), B (0.42 mg/kg), and C (0.823 mg/kg) because fluoride is essential for the normal growth of rats (Schartz and Milne 1972, Krishnamachari, 1987). The present study shows that optimal performance in bodyweight, organ/bodyweight ratio and organ weight occurs when wistar rats are exposed to fluoride at (0.823 mg/Kg) or 10 mg/l in drinking water.

Exposure to high fluoride levels in rats of groups F receiving 80 ppm (7.804 mg/kg), group E receiving 60 ppm (5.45 mg/kg) and 30 ppm (2.667 mg/kg), resulted in weights lower or equal to the control rats possibly due to enzyme inhibition. Similar results were obtained by Harrison *et al.*, (1984), where high fluoride levels inhibited body growth and reduced survival rates in growing rats. Chronic high fluoride ingestion interferes with Calcium ion transport across renal tubule endoplasmic reticulum and plasma membranes, and reduces the amount of endoplasmic reticulum and plasma membranes Calcium ion pump protein present in the kidney membranes of rat (Borke and Whitford, 1999). However, the details of the mechanism of toxicity remains unclear (Ammitzboll, *et al.*, 1988). In this study, rats exposed to 2 % tea were basically the same as the control except that they had a significant increase in kidney weight possibly due to functional hypertrophy. Dental fluorosis has been reported to be significantly correlated with the consumption of tea with milk (Cao *et al.*, 1999).

Generally muscle tissues accummulate little amounts of fluoride as compared to bone and incisor teeth in the rats. In fish, fillet was found to contain relatively low levels of fluoride as compared to osseous and cartilaginous tissues (Gikunju *et al.*, 1992). Fluoride levels in muscles of rat were significantly different from fluoride concentration in bone and teeth in all the groups investigated. Lower jaw-bone accumulates more fluoride than the femur and the incisor teeth tissues. The high fluoride levels in cancellous bone (jaw-bone) compared to compact bone (femur) were also observed by Whitford *et al.*, (1979b). Rat femur fluoride levels of 534 ± 16 mg/kg have been reported by Whitford (1991) while studying fluoride metabolism from natural ingredients and semi-purified diet. In this study, the fluoride levels in rat femur were 730.5 mg/kg, which was higher than the levels report by Whitford (1991). The disparity was probably due to different experimental approach in the two studies. The apparent similarity between fluoride in femur and incisor teeth may require further investigation.

Faecal fluoride levels vary depending on plasma fluoride levels and concentration of calcium in the diet. In this study, dietary calcium consumption was constant. The faecal fluoride levels encountered in this study were comparable to those reported by Whitford (1991) although he used intraperitoneal and oral routes of fluoride administration. The dose of fluoride administered did not influence faecal fluoride excretion significantly ($p>0.05$), although feacal excretion of fluoride increased as the dose administered increased. This was probably due to low doses used or due to poor release of fluoride from the tea and mineral salt.

In this study, sodium fluoride in drinking water was shown to induce dose related toxicity in the liver, lung, kidney and heart. There was minimal toxic effects at doses less than 30 mg/l, while 60 and 80 mg/l caused histopathological changes in the liver, lung, heart and kidney. The LD_{50} values of sodium fluoride when administered orally to rats had been reported as 36mg F/kg, (Shourie *et al.*, 1950), 86 mg/kg, (Whitford *et al.*, 1987), and 98 mg/kg, (Gruninger *et al.*, 1988). The differences in the lethal doses could be due to the various backgrounds of the experiments. Magadi mineral salt and commercial cattle salt were found to be lethal to rats when fed at 2 % solution in drinking water. Phillips *et al.*, (1934) and Kick *et al.*, (1935), reported hyalination, hydropic and fatty degeneration in the calf and pig organs after receiving rock phosphate. In this study similar tissue lesions were observed after rats were exposed to high fluoride levels. Magadi salt contributes significantly to high prevalence and severity of dental fluorosis in humans in Tanzania (Awadia *et al.*, 1999) and (Mabelya *et al.*, 1999). Although mineral supplements are used in dairy cattle, they may adversely influence the health and productivity of dairy animals due to presence of excess fluoride levels.

Histopathology showed that the swellings found in some rats were: adenoma, fibroma and a granuloma. The reason for their occurrence is obscure, although such lesions have been reported to occur spontaneously (Boorman *et al.*, 1990). Although the significance of the latter observation for human and animal safety is unknown it is recommended that excessive continuous exposure of fluoride should be avoided and further investigation should be done using more rats.

CHAPTER SIX

GENERAL DISCUSSION AND CONCLUSIONS

6.1 General discussion

Fluoride concentrations in biological, clinical and industrial samples differ due to nature of sample, method adopted for determination and level of contamination (Dabeka *et al.*,1979, Singer and Ophaug, 1979 and Venkateswarlu, 1975). Several sample characteristics may influence the fluoride detected, for instance the presence of excessive amounts of ions which bind fluoride like aluminium and calcium. Although analysis of cattle feedstuff has been done by several investigators, few have analysed mineral supplements and soup samples.

The tolerance level of fluoride in drinking water for dairy cows is 3 - 6 mg /L (Vikoren, 1995). The concentrations depend on the amount of water consumed; the lower limit being applicable for active animals in a warm climate. Most of the fluoride concentrations encountered in water in this study were below the tolerance level. However adverse productivity has been reported in dairy animals consuming as low as 2.15 mg/l in drinking water. The tolerance concentrations of fluoride are too high and should be adjusted downwards (Krook, 1998). There was a significant difference between fluoride concentration in rain and borehole water. Hence, borehole water needs constant surveillance because it can substantially increase total fluoride ingested and adversely influence milk production.

Fluoride concentration in milk was fairly constant during the wet and the dry season while urine and water fluoride concentration was significantly influenced by the season probably due to climatic factors. High temperatures tend to increase water intake and also concentrate more fluoride due to evaporation. Rainfall dilutes the water fluoride concentrations particularly in surface waters. This leads to excretion of urine of high fluoride concentration during the dry season when temperature are high and vice versa. The fluoride concentration in milk is derived from plasma fluoride. Plasma fluoride is poorly transferred to milk even when drinking water has high fluoride concentrations due to pharmacokinetic characteristics of fluoride.

Some mineral salts (e.g. Magadi salt) contain high concentrations of fluoride and may cause poor milk production. Mineral salts contained excessive concentrations of fluoride by up to about 5 times the recommended concentrations in dairy cattle feeds. Although the maximum safe level of fluoride is about 30 ppm, the essential concentrations of fluoride in dairy cattle are not known (Fraser 1986). Furthermore, there are no standards in Kenya which can be used, as guidelines in mineral mixtures for supplementing dairy cattle and feed manufacturers are likely to take advantage of the situation to the detriment of the dairy industry. The data in this study concur with other findings (Omore *et al.*, 1999) that there is low milk production in Kiambu and Thika districts dairy cattle, probably due to poor nutrition and excessive fluoride intake.

Intake of 250 ml (medium size family cup) of fish soup would provide 1.6 mg F, which is just about the minimum safe and adequate daily fluoride intake in

adults (1.5-4.0 mg F). In children, the recommended daily intake is about 0.06 mg F/kg body weight, but an increase to 0.1 mg F/kg body weight may cause dental fluorosis (National Academy of sciences, 1990). Nevertheless, consumption of 250 ml of spinach soup (and other vegetable soups) by children will only make a moderate contribution to the amount capable of causing fluorosis. Freshly prepared fish soup (fish with skin and bones) may be nutritious and rich but it could contribute significantly to increasing the prevalence of dental fluorosis particularly in children. This may increase the fluoride intake considerably. On the other hand, the reported mean values (0.1333 – 5.0111mg/L) give the total fluoride in the soup. There is great uncertainty regarding the bioavailability of fluoride in most foods (Rao, 1984). Probably only a fraction of the F in the soup will be absorbed through the gastrointestinal tract.

As the exposure dose of fluoride was increased the mean weight of rats generally increased from Control (0 mg/l) up to 10 mg/l. Exposure to high concentrations of fluoride lead to a decrease in mean body weight except for the rats fed on tea (2 % solution). Therefore rats can tolerate up to 10 mg/l without any fatalities suggesting optimal performance in body weight and organ weight. Faecal excretion of fluoride was found to increase with time in all groups of rats.

Generally muscle tissues accumulate little amounts of fluoride as compared to bone and incisor tissues in the rats. In fish, fillet contained relatively low fluoride as compared to osseous and cartilaginous tissues, (Gikunju *et al.*, 1992). Fluoride concentrations in muscles of rat were significantly different from fluoride concentration in bone and teeth in all the groups of rats investigated. In this study,

it was observed that lower jaw bone accummulates more fluoride than the femur and the incisor teeth tissues. The reason for the differences in fluoride accumulation is not well understood but the rich blood supply and comparatively large surface area of bone crystalline in cancellous bone (lower jaw) may account for the high fluoride accumulation. The distribution and density of osseus cells such as osteoblasts and osteoclasts may play a role in influencing fluoride accumulation. In the incisor teeth the distribution of ameloblasts and odontoblasts may also influence fluoride accumulation. However further investigation of the molecular basis of fluoride accumulation in different tissues may be a viable area for research.

In this study, sodium fluoride in drinking water was shown to induce dose-related toxicity in the liver, lung, kidney and heart. There were minimal toxic effects at doses less than 30 mg/l, while 60 and 80 mg/l caused obvious histopathological changes in the liver, lung, heart and kidney. Feed and water consumption remained fairly constant. The occurrence of some tumors in control and treated rats was most probably spontaneous and not related to dose of fluoride administered. A similar report on spontaneous tumours in the rat was reported by Boorman *et al.*, (1990).

6.2 Conclusions

1) The analytical method used in this study is suitable for determination of fluoride in water, soup, food substances and animal feeds.

2) Tilapia fish should not be overcooked (over 30 min) because overcooking may increase fluoride concentrations in the soup. Further investigation should be carried out on bioavailability of fluoride from fish soup.

3) This study confirms that there is low milk production in Kiambu and Thika district dairy cattle. However, there was no significant relationship between the low milk yield and fluoride concentration found in water and feedstuffs.

4) Commercial mineral salts contain more fluoride than other feeds hence their usage should be under surveillance to avoid over exposure of fluoride in dairy cattle.

5) The fluoride concentrations excreted through urine presents a better indicator for fluoride exposure in dairy cattle than milk fluoride concentrations.

6) This study has shown that fluoride is essential for normal growth of rats and the optimal fluoride intake in drinking water is 10 ppm.

7) This study confirms that cancellous bone (jaw bone) accumulate more fluoride than compact bone (femur) and other soft tissues.

8) This study has shown that fluoride concentrations above 10 ppm in drinking water causes a dose related toxicity in the liver, kidney, lungs and heart of wistar rats.

9) Rats may develop spontaneous tumours in control as well as those exposed to fluoride in drinking water, particularly in older ones (2yrs). Further investigation should be done to establish whether fluoride is tumorigenic in rats.

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APPENDICES

Appendix 1. Fluoride research questionnaire

Date .....

Sample No. .....

Area/Physical address .....

Season of the year .....

Farmer's Name and address: .....

Breed of dairy cow .....

No. of Dairy animals: .....

Concentrates used .....

Salt or mineral lick .....

Source of water (Borehole, Rain, Tap, Dam) specify .....

Source of feed: .....

Milk yield (kg) .....

Any other comments .....

Appendix 2. Composition of TISAB III

Ammonium Acetate

Ammonium Chloride

CTDA (1,2 cyclohexylene dinitrilo tetra acetic acid)

Acetic Acid

Appendix 3. Unit relationships used in fluoride concentrations

1) 1 g = 1,000 mg or 10^6 μg

0.1 g = 100 mg or 10^5 μ g

0.01 g = 10 mg or 10^4 μ g

0.001 g = 1 mg or 10^3 μ g

2) 1 mg = 1,000 μ g or 10^{-3} g

0.1 mg = 100 μ g or 10^{-4} g

0.01 mg = 10 μ g or 10^{-5} g

0.001 mg = 1 μ g or 10-6 g

3) 1 mg/Kg diet = 0.0001 %

10 mg/Kg diet = 0.001 %

100 mg/Kg diet = 0.01 %

1000 mg/Kg diet = 0.1 %

10,000 mg/Kg diet = 1 %

100,000 mg/Kg diet = 10 %

1,000,000 mg/Kg diet = 100 %

Appendix 4. Fluoride recovery from vegetables, meat and fish samples

Case	Sample	Source	Level	Initial	Added	Results
1	1	1	1	0.000	20.00	19.30
2	2	1	1	0.000	30.00	27.70
3	3	1	1	0.000	40.00	39.99
4	4	1	1	0.000	50.00	48.50
5	5	1	1	0.000	60.00	58.80
6	6	1	1	0.000	70.00	66.50
7	7	1	1	0.000	80.00	72.80
8	8	1	1	0.000	90.00	81.00
9	9	1	1	0.000	100	950.00
10	10	1	1	0.000	110	106.00
11	11	1	2	0.000	120.00	122.20
12	12	1	2	0.000	130.00	125.30
13	13	1	2	0.000	140.00	124.60
14	14	1	2	0.000	150.00	135.00
15	15	1	2	0.000	160.00	143.00
16	16	1	2	0.000	170.00	161.50
17	17	1	2	0.000	180.00	189.20
18	18	1	2	0.000	190.00	175.40
19	19	1	2	0.000	200.00	183.20
20	20	1	2	0.000	210	194.70
21	21	5	3	0.000	220.00	205.90
22	22	5	3	0.000	230.00	225.60
23	23	5	3	0.000	240.00	210.00
24	24	5	3	0.000	250.00	240.80
25	25	5	3	0.000	260.00	263.90
26	26	5	3	0.000	270.00	263.00
27	27	5	3	0.000	280.00	266.80
28	28	5	3	0.000	290.00	305.70
29	29	5	3	0.000	300.00	268.50
30	30	5	3	0.000	310.00	280.60
31	31	2	1	0.1000	0.1000	0.19
32	32	2	1	0.1100	0.2000	0.29
33	33	2	1	0.0900	0.3000	0.38
34	34	2	1	0.1200	0.4000	0.49
35	35	2	1	0.1400	0.5000	0.60

Appendix 4 (cont)

Case	Sample	Source	Level	Initial	Added	Results
36	36	2	1	0.1600	1.000	1.04
37	37	2	1	0.1300	2.000	1.94
38	38	2	1	0.1700	3.000	3.20
39	39	2	1	0.1100	4.000	3.97
40	40	2	1	0.1000	5.000	5.00
41	41	3	1	0.2100	0.1000	0.27
42	42	3	1	0.2500	0.2000	0.41
43	43	3	1	0.2700	0.3000	0.57
44	44	3	1	0.2000	0.4000	0.56
45	45	3	1	0.2400	0.5000	0.73
46	46	3	1	0.2600	100.00	1.10
47	47	3	1	0.2800	200.00	2.10
48	48	3	1	0.3000	2.6000	2.60
49	49	3	1	0.2500	2.7000	2.80
50	50	3	1	0.3000	300.00	3.40
51	51	4	1	17.000	1.5000	14.50
52	52	4	1	20.000	2.1000	18.90
53	53	4	1	21.000	2.4000	18.70
54	54	4	1	22.000	2.5000	16.50
55	55	4	1	19.000	1.6000	17.90
56	56	4	1	18.000	1.8000	15.00
57	57	4	1	230.00	200.00	22.80
58	58	4	1	210.00	2.6000	26.00
59	59	4	1	170.00	2.7000	18.20
60	60	4	1	25.000	3.000	29.40
61	61	6	1	1.500	0.5000	1.48
62	62	6	1	0.900	0.5000	0.90
63	63	6	1	0.900	0.5000	1.10
64	64	6	1	1.400	0.5000	1.50
65	65	6	1	1.600	0.5000	1.30
66	66	6	1	1.900	0.5000	2.40
67	67	7	1	0.400	2.5000	2.80
68	68	7	1	0.300	2.5000	2.80
69	69	7	1	0.100	2.5000	2.30
70	70	7	1	0.200	2.5000	2.70
71	71	7	1	0.400	2.5000	2.50

Appendix 4 (cont)

Case	Sample	Source	Level	Initial	Added	Results
72	72	7	1	0.100	2.5000	2.30
73	73	8	1	0.200	2.5000	2.80
74	74	8	1	0.100	2.5000	2.50
75	75	8	1	0.400	2.5000	2.60
76	76	8	1	0.300	2.5000	2.80
77	77	8	1	0.300	2.5000	2.40
78	78	8	1	0.600	2.5000	2.80
79	79	9	1	0.700	0.5000	0.90
80	80	9	1	1.200	0.5000	1.70
81	81	9	1	0.800	0.5000	1.20
82	82	9	1	1.000	0.5000	1.10
83	83	9	1	0.800	0.5000	1.00
84	84	9	1	0.900	0.5000	1.20
85	85	10	1	0.500	1.5000	2.20
86	86	10	1	0.800	1.5000	2.00
87	87	10	1	0.600	1.5000	2.00
88	88	10	1	0.400	1.5000	1.70
89	89	10	1	0.500	1.5000	2.10
90	90	10	1	0.300	1.5000	1.98
91	91	11	1	0.500	1.5000	2.10
92	92	11	1	0.300	1.5000	1.60
93	93	11	1	0.400	1.5000	1.70
94	94	11	1	0.200	1.5000	1.90
95	95	11	1	0.300	1.5000	1.60
96	96	11	1	0.100	1.5000	1.40
97	97	12	1	0.400	2.5000	2.30
98	98	12	1	0.600	2.5000	2.60
99	99	12	1	0.500	2.5000	2.10
100	100	12	1	0.300	2.5000	2.10
101	101	12	1	0.400	2.5000	2.30
102	102	12	1	0.200	2.5000	2.10
103	103	13	1	0.600	1.5000	2.20
104	104	13	1	0.700	1.5000	2.10
105	105	13	1	0.500	1.5000	1.90
106	106	13	1	0.800	1.5000	2.20
107	107	13	1	0.300	1.5000	1.90

Appendix 4 (cont)

Case	Sample	Source	Level	Initial	Added	Results
108	108	13	1	0.700	1.5000	2.30
109	109	14	1	0.900	1.5000	2.50
110	110	14	1	0.800	1.5000	2.20
111	111	14	1	0.800	1.5000	2.50
112	112	14	1	0.900	1.5000	2.50
113	113	14	1	1.000	1.5000	2.50
114	114	14	1	1.000	1.5000	2.40

KEY:

SOURCE:

1=blank tube, 2=bran, 3=concentrate (dairy meal), 4=mineral salt, 5=blank II solution, 6=Lettuce, 7=kales, 8=Cabbage, 9=Spinach, 10=Bovine meat, 11=Broiler meat, 12=Goat meat, 13=Nile perch, 14=Nile Tilapia

LEVEL:

1=low addition of fluoride, 2=medium addition of fluoride, 3=high addition of fluoride, 4=mineral salt

Appendix.5 Fluoride concentration (ppm) in vegetables, meat and fish soup upon boiling.

Case	Time*	Control	Bovine	Goat	Chicken	Tilapia	N/perch	Cabbage	Kales	Lettuce	Spinach
1	0.00	0 .14	0.23	0.17	0.12	2.50	2.30	0.18	0.22	0.26	0.42
2	15.0	0.13	0.24	0.17	0.13	3.70	2.40	0.19	0.22	0.33	0.43
3	30.0	0.12	0.27	0.19	0.14	4.40	2.50	0.21	0.22	0.45	0.46
4	45.0	0.12	0.32	0.20	0.15	5.10	2.60	0.24	0.24	0.52	0.49
5	60.0	0.11	0.34	0.21	0.15	5.40	2.70	0.27	0.25	0.62	0.60
6	75.0	0.13	0.36	0.23	0.17	5.40	3.10	0.28	0.25	0.65	0.66
7	90.0	0.14	0.37	0.26	0.18	5.90	3.30	0.30	0.27	0.80	0.70
8	105.0	0.15	0.38	0.29	0.20	6.30	3.60	0.31	0.28	1.10	0.89
9	120.0	0.16	0.38	0.28	0.23	6.40	3.80	0.31	0.29	1.30	1.30

- Time was recorded in minutes

Appendix 6. Fluoride concentration in animal feeds from Kiambu and Thika districts

FARMER	AREA	SOCIETY	BREED	SAMPLE	FLUORIDE(mg/kg)
A. Gathethe	Magumu	Chania	Jersey	mineral salt	19
E Wambui	Ngaa	Chania	Fresian	bran	48
E. Kirori	Bangoro	Chania	Aryshire	bran	17.5
F. Gachera	Mugerere	Chania	Fresian	bran	37.9
Fn gachera	Mugerere	Chania	Fresian		
G kaage	Kiamwere	Chania	Fresian	mineral salt	35
G kaage	Kiamwere	Chania	Aryshire	mineral salt	302.3
G kaage	Kiamwere	Chania	Fresian	bran	48.7
K njoroge	Ngorongo	Chania	Fresian	maize stalk	14.69
M kariri	Mangu	Chania	Fresian		
Nj margaret	Kahuroko	Chania	Fresian	mineral salt	29.3
Nj margaret	Kahuroko	Chania	Fresian	napier grass	12.4
Nj margaret	Kahuroko	Chania	Fresian	Napier grass	12.5
Mn mbugua	Gatei	Chania	Fresian	Dairy meal	28.4
Mn mbugua	Gatei	Chania	Fresian		
Mw mwangi	Main dairy	Chania	Fresian	mineral salt	165
Pw kahubi	Nyamathumbi	Chania	Aryshire	mineral salt	125.5
Pw kahubi	Nyamathumbi	Chania	Aryshire	bran	16.1
S mbogo	Gatunguru	Chania	Fresian	dairy meal	31.8
S wanjiku	Gituamba	Chania	Aryshire	wheat bran	23.8
Tn waweru	Mugerere	Chania	Fresian	wheat	25.3
A mbiyu	Turitu	Kiambaa	Fre/ary		
A mbiyu	Turitu	Kiambaa	Fre/ary		
A mbiyu	Turitu	Kiambaa	Fre/ary	mineral salt	83.3
A mbiyu	Turitu	Kiambaa	Fre/ary	napier grass	16.17
Fw njoroge	Mucatha	Kiambaa	Fresian	maize germ	9.7
Fw njoroge	Mucatha	Kiambaa	Fresian		
Fw njoroge	Mucatha	Kiambaa	Fresian		
Fw njoroge	Mucatha	Kiambaa	Fresian	mineral salt	473.6
Jm njogu	Karura	Kiambaa	Jersey	bran	32.7
Jm njogu	Karura	Kiambaa	Aryshire		
Jw kinyanju	Karura	Kiambaa	Fresian		
Jw kinyanju	Karura	Kiambaa	Fresian		
Jw kinyanju	Karura	Kiambaa	Fresian		
Jw kinyanju	Karura	Kiambaa	Fresian		
Jw kinyanju	Karura	Kiambaa	Fresian	dairy meal	61.4
Jw kinyanju	Karura	Kiambaa	Fresian		
Jw kinyanju	Karura	Kiambaa	Fresian		
Jw kinyanju	Karura	Kiambaa	Fresian		
Jw kinyanju	Karura	Kiambaa	Fresian		
Jw kinyanju	Karura	Kiambaa	Fresian		
Jw kinyanju	Karura	Kiambaa	Fresian		
K gicinju	Gathanga	Kiambaa	Aryshire		
K gicinju	Gathanga	Kiambaa	Aryshire		
K githarie	Karura	Kiambaa	Aryshire	dairy meal	31.1

Appendix 6 (cont)

FARMER	AREA	SOCIETY	BREED	SAMPLE	FLUORIDE(mg/kg)
Lw gathuri	Gathanga	Kiambaa	Fresian	maize stalk	12.89
Lw gathuri	Gathanga	Kiambaa	Fresian		
Lw gathuri	Gathanga	Kiambaa	Fresian		
Lw kahoro	Githanga	Kiambaa	Aryshire		
Lw kahoro	Githanga	Kiambaa	Guernsey	dairy meal	6.9
Lw kahoro	Githanga	Kiambaa	Aryshire		
Lw muiruri	Riara ridg	Kiambaa	Fresian		
P m muigai	Route 10	Kiambaa	Fresian		
Pm kinyanju	Karura	Kiambaa	Fresian		
Pm kinyanju	Karura	Kiambaa	Fresian	bran	29.6
Pm kinyanju	Karura	Kiambaa	Fresian		
Pm kinyanju	Karura	Kiambaa	Fresian	magadi soda	1051.5
Pm kinyanju	Karura	Kiambaa	Fresian		
Pm kinyanju	Karura	Kiambaa	Fresian		
Pm kinyanju	Karura	Kiambaa	Fresian		
Pm kinyanju	Karura	Kiambaa	Fresian		
Pm kinyanju	Karura	Kiambaa	Fresian	dairy meal	31.6
Sk mbugua	Route1	Kiambaa	Fresian		
Sk mbugua	Route1	Kiambaa	Fresian	mineral salt	31.2
Sm njoroge	Route 1	Kiambaa	Aryshire		
Sm njoroge	Route 1	Kiambaa	Aryshire		
Sw karanja	Riararidge	Kiambaa	Fresian	dairy meal	18
Sw karanja	Riararidge	Kiambaa	Fresian	dairy meal	17.3
Sw karanja	Riararidge	Kiambaa	Fresian	maize germ	16
Sw karanja	Riara ridg	Kiambaa	Aryshire	dairy meal	9.2
Sw karanja	Riara ridg	Kiambaa	Aryshire	bran	7.8
Sw karanja	Riara ridg	Kiambaa	Aryshire	maize germ	10.5
Sw karanja	Riara ridg	Kiambaa	Aryshire	bran	5.8
Tw warukira	Gathanga	Kiambaa	Fresian	bran	21.6
Tw warukira	Gathanga	Kiambaa	Fresian	mineral salt	48.3
Tw warukira	Gathanga	Kiambaa	Fresian	mineral salt	173.4
Tw warukira	Gathanga	Kiambaa	Fresian	maize germ	7.1
Dw wambaa	Muthiga	Kikuyu	Guernsey		
Dw wambaa	Muthiga	Kikuyu	Guernsey	maize germ	10.9
I wanjiru	Kikuyu	Kikuyu	Fresian		
J wambui	Kinoo	Kikuyu	Fresian		
J wambui	Kinoo	Kikuyu	Canine	dog feed	28.7
J wambui	Kinoo	Kikuyu	Fresian	dairy meal	13.8
J wambui	Muthiga	Kikuyu	Fresian	themeda triadra	5.5
Kw kamau	Ondiri	Kikuyu	Fresian	dairy meal	18.5
Kw kamau	Ondiri	Kikuyu	Fresian		
Kw kamau	Ondiri	Kikuyu	Fresian	maize germ	15.3
Kw kamau	Ondiri	Kikuyu	Fresian		
M wanjuhi	Muthiga	Kikuyu	Fresian	maize germ	11.7
M wanjuhi	Muthiga	Kikuyu	Fresian	saw dust-c	17.6
M wanjuki	Kikuyu	Kikuyu	Fresian		
Mw kimari	Muthiga	Kikuyu	Fresian		

Appendix 6 (cont)

FARMER	AREA	SOCIETY	BREED	SAMPLE	FLUORIDE(mg/kg)
Mw kimari	Muthiga	Kikuyu	Fresian		
Njoroge	Kahuho	Kikuyu	Aryshire	wheat bran	33.89
Njoroge	Kahuho	Kikuyu	Aryshire	maize germ	32.73
P nyacira	Kiambaa	Kikuyu	Aryshire		
P nyacira	Kiambaa	Kikuyu	Aryshire		
Pw regild	Muthiga	Kikuyu	Fresian		
Pw regild	Muthiga	Kikuyu	Fresian		
Pw regild	Muthiga	Kikuyu	Fresian		
Pw regild	Muthiga	Kikuyu	Fresian		
R wanjiru	Muthiga	Kikuyu	Fresian	napier grass	9.7
Rw chege	Kawangware	Kikuyu	Fresian		
Rw chege	Kawangware	Kikuyu	Fresian	themeda triadra	12.8
Rw chege	Kawangware	Kikuyu	Fresian	chicken manure	19.7
Rrw chege	Kawangware	Kikuyu	Fresian	themeda triadra	7.8
Rrw chege	Kawangware	Kikuyu	Fresian		
Rrw chege	Kawangware	Kikuyu	Fresian	calf pellet	15.6
Rw chege	Kawangware	Kikuyu	Fresian		
Rw chege	Kawangware	Kikuyu	Fresian		
Rw chege	Kawangware	Kikuyu	Fresian		
Sn mukuria	Kikuyu	Kikuyu	Aryshire		
Sn mukuria	Kikuyu	Kikuyu	Aryshire		
Sn mukuria	Kikuyu	Kikuyu	Aryshire		
Sn mukuria	Kikuyu	Kikuyu	Aryshire		
Sn mukuria	Kikuyu	Kikuyu	Aryshire		
Sn mukuria	Kikuyu	Kikuyu	Aryshire		
Sn mukuria	Kikuyu	Kikuyu	Aryshire		
Ssn mukuria	Kikuyu	Kikuyu	Aryshire		
V thidau	Kanyariri	Kikuyu	Fresian	chicken manure	19.64
V thidau	Kanyariri	Kikuyu	Fresian		
Vg gitau	Kahuho	Kikuyu	Fresian	mineral salt	145.45
Vg gitau	Kahuho	Kikuyu	Fresian	maize germ	43.51
Vg gitau	Kahuho	Kikuyu	Fresian		
W mwaniki	Kahuho	Kikuyu	Fresian	maize germ	18.12
W mwaniki	Kahuho	Kikuyu	Fresian		
W mwaniki	Kahuho	Kikuyu	Fresian		
W mwaniki	Kahuho	Kikuyu	Fresian		
Wairimu	Uthiru	Kikuyu	Fresian	maize germ	18.8
Wairimu	Uthiru	Kikuyu	Fresian	dairy meal	13.1
Wairimu	Uthiru	Kikuyu	Fresian	mineral salt	17.9
Wm kimari	Muthiga	Kikuyu	Aryshire		
Ek kihoro	Uplands	Lari	Aryshire		
Ek kihoro	Uplands	Lari	Aryshire		
G kanduma	Up hill	Lari	Aryshire	bran	23.7
G kanduma	Up hill	Lari	Aryshire	mineral salt	443.5
G kanduma	Up hill	Lari	Aryshire		
G kanduma	Up hill	Lari	Aryshire		
Jn kimani	Uplands	Lari	Fresian		
K kamau	Up hill	Lari	Jersey		

Appendix 6 (cont)

FARMER	AREA	SOCIETY	BREED	SAMPLE	FLUORIDE(mg/kg)
K karanja	Accr\tarmc	Lari	Fresian	comellina	16.4
K muariama	A/railway	Lari	Fresian		
K muariama	A/railway	Lari	Fresian		
K muariama	A/railway	Lari	Fresian		
K mwangi	Uplands	Lari	Fresian	mineral salt	125.5
K mwangi	Uplands	Lari	Fresian		
K mwangi	Uplands	Lari	Fresian		
K mwangi	Uplands	Lari	Fresian		
K mwangi	Uplands	Lari	Fresian	mineral salt	574.5
M gitundu	Uplands mk	Lari	Fresian		
M gitundu	Uplands mk	Lari	Fresian		
M gitundu	Uplands mk	Lari	Fresian		
M kagari	Accr/rail	Lari	Jersey		
M kimani	Accr\tarmc	Lari	Guernsey		
M kimani	Accr\tarmc	Lari	Guernsey		
M ngugi	A/railway	Lari	Aryshire		
M ngugi	A/railway	Lari	Aryshire		
Mn gichana	Uplands	Lari	Fresian		
Mn gichana	Uplands	Lari	Fresian	Brewers waste	37
N kabuchi		Lari	Fresian		
N kabuchi		Lari	Fresian		
N kamau	Accr/tarmc	Lari	Fresian		
N kamau	Accr/tarmc	Lari	Fresian		
N mundati	Dfc siderd	Lari	Guernsey		
N mundati	Dfc siderd	Lari	Guernsey		
N mundati	Dfc siderd	Lari	Guernsey		
Vm gichanga	Uplands mk	Lari	Guernsey		
C mburu	Gg's house	Limuru	Aryshire	maize germ	28.7
C mburu	Gg's house	Limuru	Aryshire		
C mburu	Gg's house	Limuru	Aryshire	bran	27.5
C mburu	Gg's house	Limuru	Aryshire		
C mburu	Gg's house	Limuru	Aryshire		
C mburu	Gg's house	Limuru	Aryshire	pyrethrum wate	32
G gathuru	Rironi	Limuru	Aryshire		
G kagunda	Ngecha	Limuru	Fresian	mineral salt	242.5
G kagunda	Ngecha	Limuru	Fresian	mineral salt	8.7
G kagunda	Ngecha	Limuru	Fresian	maize germ	22.4
G kagunda	Ngecha	Limuru	Fresian		
H ngugi	Ngecha	Limuru	Fre/ary	maize germ	9.4
H ngugi	Ngecha	Limuru	Aryshire	mineral salt	219.7
H ngugi	Ngecha	Limuru	Aryshire		
H ngugi	Ngecha	Limuru	Aryshire		
H ngugi	Ngecha	Limuru	Aryshire		
H ngugi	Ngecha	Limuru	Aryshire		
I mwaniki	Tiekunu	Limuru	Fresian	mineral salt	273
I mwaniki	Tiekunu	Limuru	Fresian	bran	7.9
I mwaniki	Tiekunu	Limuru	Fresian	napier grass	12
I mwaniki	Tiekunu	Limuru	Fresian	maize stalk	

Appendix 6 (cont)

FARMER	AREA	SOCIETY	BREED	SAMPLE	FLUORIDE(mg/kg)
I mwaniki	Tiekunu	Limuru	Fresian	maize stalk	12.3
Im njuguna	Tiekunu	Limuru	Fresian		
J muhia	Mirithu	Limuru	Fresian	chicken manure	57.5
J muhia	Mirithu	Limuru	Fresian		
Jn njoroge	Kerwa	Limuru	Aryshire		
N kaigwa	Rironi	Limuru	Aryshire		
P njenga	Murengeti	Limuru	Fresian	maize germ	17.9
P njenga	Murengeti	Limuru	Fresian		
Pk giathi	Murengeti	Limuru	Fresian		
Pk giathi	Murengeti	Limuru	Fresian		
Pk giathi	Murengeti	Limuru	Fresian		
Pm gichua	Ngecha	Limuru	Fresian		
W kimemia	Murengeti	Limuru	Guernsey		
W njoroge	Murengeti	Limuru	Fresian	wheat bran	42
W njoroge	Murengeti	Limuru	Fresian		
Aw micwe	Hamindo	Nderi	Fresian	star grass	23
Aw mwai	Hamindo	Nderi	Fresian		
Gk gaturu	Karai	Nderi	Fresian	maize germ	21.96
Gk gaturu	Karai	Nderi	Fresian	dairy meal	7.3
Gm mugane	Hamindo	Nderi	Jersey	dairy cubes	15.6
Gm mugane	Hamindo	Nderi	Jersey	dairy meal	9.2
Gm mugane	Hamindo	Nderi	Jersey		
Gm mugane	Hamindo	Nderi	Jersey		
Gw ngugi	Gitaru	Nderi	Aryshire	dairy meal	35.1
Gw ngugi	Gitaru	Nderi	Aryshire		
Gw ngugi	Gitaru	Nderi	Aryshire		
Jc nganga	Gitaru	Nderi	Fresian		
Jc nganga	Gitaru	Nderi	Fresian		
Lm Muigai	Karai	Nderi	Fresian	bran	
Lm muigai	Karai	Nderi	Fresian	chicken manure	46.6
Lm muigai	Karai	Nderi	Fresian		
Lm muigai	Karai	Nderi	Fresian	dairy meal	9.7
Lw waweru	Hamindo	Nderi	Jersey	dairy meal	18.94
Lw waweru	Hamindo	Nderi	Jersey	bran	34.21
Lw waweru	Hamindo	Nderi	Jersey	dairy meal	22.9
Mn ndungu	Gitaru	Nderi	Aryshire		
Mn ndungu	Gitaru	Nderi	Aryshire		
Mn kuria	Hamindo	Nderi	Fresian	bran	11.8
Mt kibiro	Hamindo	Nderi	Fresian		
Mt kibiro	Hamindo	Nderi	Fresian	maize germ	27.2
Mw gatimu	Gitaru	Nderi	Fresian		
Mw gatimu	Gitaru	Nderi	Fresian	bran	8.4
Mw gatimu	Gitaru	Nderi	Fresian	Brewers waste	21
Mw gatimu	Gitaru	Nderi	Fresian	maize germ	5.63
Mw gatonye	Kamuguga	Nderi	Fresian		
Ndirangu	Gitaru	Nderi	Fresian	dairy meal	30.73
Sn githu	Hamindo	Nderi	Fresian		
Sn githu	Hamindo	Nderi	Fresian		

Appendix 6 (cont)

FARMER	AREA	SOCIETY	BREED	SAMPLE	FLUORIDE(mg/kg)
Sa githu	Hamindo	Nderi	Fresian		
Sa githu	Hamindo	Nderi	Fresian		
Sa githu	Hamindo	Nderi	Fresian		
Sa githu	Hamindo	Nderi	Fresian		
Sa githu	Hamindo	Nderi	Fresian		
Sa githu	Hamindo	Nderi	Fresian	themeda triadra	8.4
Sa githu	Hamindo	Nderi	Fresian	dairy meal	13.7
Sa githu	Hamindo	Nderi	Fresian		
Sa githu	Hamindo	Nderi	Fresian		
Va kamau	Karai	Nderi	Fresian		

Appendix. 7. Fluoride concentrations (ppm) in water and milk samples

WATERSOURCE	WATERF	WATERF1	MILKF	MILKF1	URINEF	URINEF1
Tap	0.17	0.19	0.035		1.2	
Tap	0.28	0.04	0.04		1.5	
Rain	0.145		0.04		1.2	
Tap	0.215		0.03		1.6	
Tap	0.215		0.03		1.6	
Rain	0.13		0.034	0.05	0.66	
Rain	0.12	0.02	0.032	1.6	3.8	
Tap	0.215		0.025		0.9	
Rain	0.23		0.02		0.72	
Tap	0.22	0.11	0.03	0.01	1	
Tap	0.125	0.11	0.06		1.6	
Rain	0.118	0.045	0.027	0.041	1.2	
Tap	0.12	0.02	0.055		1.4	
Rain	0.046		0.39		0.8	
Rain	0.1	0.056	0.02	0.024	0.8	
Tap/rain	0.2	0.07	0.035	0.044	0.9	0.05
Tap/rain	0.2	0.07	0.035	0.044	0.9	0.05
Borehole	0.29	0.3	0.04	0.05	2	
Tap	0.12		0.038		1.6	
Borehole	0.37		0.036		2	
Tap	0.175	0.05	0.03	0.052	1	
Tap	0.15	0.085	0.04	0.056	1.3	0.034
Tap	0.12	0.11	0.04	0.054	2.4	
Tap	0.12	0.11	0.04	0.05	2.4	
Rain	0.19		0.03		1.2	
Borehole	0.13		0.022		1.5	3
Tap	0.19	0.02	0.039	0.035	1.5	
Dam	0.2	0.11	0.039	0.04	1.3	0.9
Dam/tap	0.2	0.11	0.039	0.04	1.3	0.9
	0.19					
	0.19					
Borehole	0.26	0.054	0.04	0.03	1.8	
Tap	0.27	0.15	0.039	0.037	2.5	
Rain	0.24	0.2	0.025	0.026	1.1	
Tap	0.028	0.11	0.036	0.05	6.8	
Borehole	0.39	0.26	0.089	0.35	3.5	
Tap	0.35	0.33	0.031		0.9	
Tap	0.35	2.8	0.03	0.15	1.4	
Tap	0.28	0.08	0.05	0.065	1.6	
Rain	0.275	0.2	0.039	0.06	0.8	
Tap	0.115	0.33	0.03	0.071	1.5	
Borehole	0.6		0.054	0.095	1.9	
Rain	0.36	0.28	0.04	0.054	1.2	
Tap	0.38	0.1	0.064		1.35	0.46
Rain	0.2	0.17	0.036	0.065	0.37	
Tap	0.265	0.21	0.045		3.4	3.4
Borehole	0.32	0.019	0.028	0.054	0.7	0.68

Appendix 7 (cont)

WATERSOURCE	WATERF	WATERF1	MILKF	MILKF1	URINEF	URINEF1
Rain	0.041	0.09	0.042	0.08	0.44	2
Rain	0.185		0.021	0.034	0.36	
Tap	0.078	0.066	0.083		0.7	
Tap	0.16	0.17	0.03	0.025	0.31	
Tap	0.2	0.11	0.029	0.038	1.2	0.16
Dam	0.018		0.024		0.68	0.76
Rain	0.22	0.28	0.036		0.4	0.45
Tap	0.13		0.016		0.042	
Borehole	0.32	0.18	0.045		0.46	
Borehole	0.2		0.03		2.3	
Tap	0.2		0.03		1.2	
Dam	0.18	0.18	0.05	0.05	1.9	
Tap	0.16	0.31	0.09		0.4	
Rain	0.15	0.072	0.03	0.1	1.9	0.2
Tap	0.058	0.21	0.04		0.9	
Tap	0.038		0.016		0.43	
Borehole	1.6	0.072	0.034	0.12	1.8	
Tap	0.2	0.21	0.034	0.12	0.6	0.52
Rain	0.023	0.21	0.039	0.14	0.5	
Tap	0.2	0.09	0.03	0.12	0.38	0.24
Tap	0.083		0.04	0.08	1.1	
Tap	0.054	0.025	0.042	0.036	1.1	
Tap	0.26		0.03		1.3	
Rain	0.044	0.082	0.04	0.08	1.6	0.55
Rain	0.02	0.24	0.04		0.33	
Tap	0.201	0.13	0.04		1.2	1.6
Tap	0.25	0.35	0.06		2.4	
Rain	0.25		0.03		0.9	
Borehole	3.2	0.03	0.062	0.055	0.66	
Dam	0.2		0.04		0.8	
Rain	0.24	0.29	0.035		2.1	
Tap	0.18	0.086	0.06		0.85	
Borehole	0.36	0.035	0.038	0.085	4.6	1.7
Borehole	0.29	0.038	0.066	0.065	1.08	0.03
Borehole	0.29	0.39	0.12	0.072	0.44	
Tap	0.24	0.28	0.034		0.7	
Rain	0.205	0.44	0.105		1.2	
Tap	0.2	0.56	0.1		0.45	
Tap	0.2	0.35	0.115		1.2	0.56
Borehole	3.4		0.052		3.9	

KEY
WATERF = fluoride levels of water samples taken during the wet season
WATERF1 = fluoride levels of water samples taken during the dry season
MILKF = fluoride levels of milk samples taken during the wet season
MILKF1 = fluoride levels of milk samples taken during the dry season
URINEF = fluoride levels of urine samples taken during the wet season
URINEF1= fluoride levels of urine samples taken during the dry season

Appendix 8. Milk production (l/day/cow) in dairy cattle from Kiambu and Thika districts over 12 visits

ANIMAL	FARMER	VM1	VM2	VM3	VM4	VM5	VM6	VM7	VM8	VM9	VM10	VM11	VM12
Kairitu	A.Gathethe	6	5	7,5	0	7	9	6,5	3,5	6	6	8	7
Maritati	E Wambui	6	5	4	2,5	4,5	5,6	2,5	2,1	1,4	0,3	0	0
Kairitu	En Kirori	4	7	5	4	5	7	4	2	4	1,4	0	0
	Fn Gachera	0	0	0	0	0	0	0	0	0	0	0	0
Kairitu	Fn Gachera	1	0	0	0	13	14,5	11	11	7	7,5	7	8
Queen	G Kaage	7	6	6	4,5	5	2	2	0,7	0	12	13	0
	G Kaage	0	0	0	0	0	0	0	0	0	0	0	0
	G Kaage	0	0	0	0	0	0	0	0	0	0	0	0
December	K Njoroge	7	0	0	0	6	0	5	7	8	8	6	3
Kabuni	M Kariri	12	9	12	6	6,5	0	0	0	0	0	0	0
Thambu	Nj Margaret	7	7	9	6,5	6	6	5	4,5	5	3	0	0
	Nj Margaret	0	0	0	0	0	0	0	0	0	0	0	0
	Nj Margaret	0	0	0	0	0	0	0	0	0	0	0	0
Kairitu	Mn Mbugua	0	0	0	0	0	0	0	0	0	0	0	6
Wahinya	Mn Mbugua	1	1	0	0	0	7	5	4	7	6	7	0
Ciringi	Mw Mwangi	4	3,5	2,5	1	0,5	1,4	0,5	0	0	6	4,5	3
Kairitu	Pw Kahubi	3	7	3	3,5	2,5	1,4	0	0	0	0	8	7
	Pw Kahubi	0	0	0	0	0	0	0	0	0	0	0	0
Mairu	S Mbogo	13	7	7	5	6	6	2	8	0	8	9	10
Meni	S Wanjiku	6	0,5	0	0	0	0	4	8	4	4,5	8	6
Ngunu	Tn Waweru	7	0	0	0	15	15	13,5	13	9	10	7,7	10
Kiheo	A Mbiyu	7	5	3	5	5	3	0	0	5	3	7	7
Lilly	AMbiyu	0	0	0	0	0	0	0	0	0	0	7	7
Hatari	A Mbiyu	5	5	3	5	5	4	2	0,5	1	3	0	11
	A Mbiyu	0	0	0	0	0	0	0	0	0	0	0	0
Jane	Fw Njoroge	2	2,1	2,5	2,5	0	0	0	0	0	0	0	0
Wasiwasi	Fw Njoroge	14	9,8	10	7	6	10	10	6	4,2	5	5	6
Nyameni	Fw Njoroge	13	4,9	3	0	0	13	14	14	4,9	9	9	7
	Fw Njoroge	0	0	0	0	0	0	0	0	0	0	0	0
	Jm Njogu	0	0	0	0	0	0	0	0	0	0	0	0
Beauty	Jm Njogu	0	4,5	0	0	12,6	8	8	4	10,5	9,8	7,5	4,9
Wf	Jw Kinyanjui	0	0	0	7	7,5	6	4	9	0	0	0	11
Farasi	Jw Kinyanjui	5	7	6	10	6	2	3	10	7	6	6	4,5
Kamary	Jw Kinyanjui	0	0	0	0	0	0	0	0	0	12	11	13
Muikamba	Jw Kinyanjui	4,5	8	9	8,5	6,5	8	6	9	5	5	6	2
BF	Jw Kinyanjui	0	0	0	22	15	10	7	17	8	10	10	9
Monica	Jw Kinyanjui	0	0	0	0	0	0	6	15	6	9	7	7,5
Bindu	Jw Kinyanjui	10	10	18	19	18	10	5	16	8	11,5	10	10,5
Kairitu	Jw Kinyanjui	0	0	0	18	9	8	6	10	6	11	10	8
Kinyanya	Jw Kinyanjui	7	9	4	5	0	0	0	0	0	0	0	0
Survivor	Jw Kinyanjui	9	0	8	14	6,5	0	0	0	0	0	0	0
Mf	Jw Kinyanjui	0	0	0	5	6	9	4	8	6	5,5	0	0
Kamugeni	K Gicinju	0	12	0	0	0	0	0	0	0	0	0	0

Appendix 8 (cont)

ANIMAL	FARMER	VM1	VM2	VM3	VM4	VM5	VM6	VM7	VM8	VM9	VM10	VM11	VM12
Ngunu	K Gicinju	10	4	0	0	0	3,5	3,5	3	3	2,8	0	0
Jemima	K Githarie	6	3,5	3,5	3	0	0	0	5	7	10	5	2
Minnie	Lw Gathuri	4,5	4	4	4,5	5	7,5	0	0	11,5	7,5	3	0
Udder	lw Gathuri	3	5	0	4,5	6	4	9	6,3	5,5	4	2,5	5
Njeri	Lw Gathuri	0	5	6	4,5	6	4	3	4,9	7,5	3,5	2	5
Mugeni	Lw Kahoro	5	5	5	3,5	2	7,5	3,5	4	7	3,5	4,5	4,5
	Lw Kahoro	0	0	0	0	0	0	0	0	0	0	0	0
Maritati	Lw Kahoro	0	0	0	5	3	7,5	4,5	4,2	7	4,9	6	4,5
Kamuri	Lw Muiruri	17	19	14	0	0	0	0	0	0	0	0	0
Kanini	P m Muigai	0	0	0	0	0	12	12,5	12	14,6	4,9	17	15
Seven	Pm Kinyanju	0	0	0	0	0	0	5	5	3	5	5,5	3
	Pm Kinyanju	0	0	0	0	0	0	0	0	0	0	0	0
Six	Pm Kinyanju	6	6,5	5,5	4	6	4,9	5	0	0	0	0	0
	Pm Kinyanju	0	0	0	0	0	0	0	0	0	0	0	0
Four	Pm Kinyanju	5	5	5	4	5	4,2	3	1	0	0	0	15
Three	Pm Kinyanju	2	1,5	1,5	2	3	3,5	2	2	2,5	3	5,5	2,5
Two	Pm Kinyanju	5	7	5	3	6	4,9	0	0	0	0	0	0
Eight	Pm Kinyanju	5	2,5	3,5	3	5	4,2	2	2	0	0	10,5	15
One	Pm Kinyanju	10	8	7,5	5	5,5	4,2	3	4,5	2	4	5	4,5
Jane	Sk Mbugua	7	6	5	3,5	3	2	2,1	2	0	0	0	0
Beatrice	Sk Mbugua	5	6	5	3,5	0,5	5,2	0	0	0	0	0	0
Birthday	Sm Njoroge	2	0	10,5	0	13	6	0	0	0	0	0	0
June	Sm Njoroge	2	0	13,5	15	13,5	9	7,5	10,5	8	7,7	6,5	7
	Sw Karanja	0	0	0	0	0	0	0	0	0	0	0	0
	Sw Karanja	0	0	0	0	0	0	0	0	0	0	0	0
	Sw Karanja	0	0	0	0	0	0	0	0	0	0	0	0
	Sw Karanja	0	0	0	0	0	0	0	0	0	0	0	0
	Sw Karanja	0	0	0	0	0	0	0	0	0	0	0	0
Jane	Sw Karanja	4	0	0	0	4	7,5	9	7,5	2	7	8,5	13,5
	Sw Karanja	0	0	0	0	0	0	0	0	0	0	0	0
	Tw Warukira	0	0	0	0	0	0	0	0	0	0	0	0
	Tw Warukira	0	0	0	0	0	0	0	0	0	0	0	0
Wanjiku	Tw Warukira	0	0	0	0	2	7	5,5	6,5	8,5	8	9	9
	Tw Warukira	0	0	0	0	0	0	0	0	0	0	0	0
Kamure	Dw Wambaa	0	15,5	7	5	4,5	3,5	5	5	5	3,5	5	4
Haraka	Dw Wambaa	5	4	4	3,5	2	0	0	11	9	5	6	5
Ngei	I Wanjiru	4,5	0	10,5	7	6	5,5	4	5	5	0,5	3	4,5
Kanini	J Wambui	3	4	1	0	0	0	12	11	8	7	7	7
Kagondo	J Wambui	10	10	9	8	9	0	0	0	0	0	0	0
Kagira	J Wambui	2	4	6	2,5	0	0	0	0	0	0	0	0
	J Wambui	0	0	0	0	0	0	0	0	0	0	0	0
Kanini	Kw Kamau	0	0	0	0	0	0	0	0	7	8	6	6
Muguga	Kw Kamau	1	0	12	14	9	11	4	9	6	5	5	4
	Kw Kamau	0	0	0	0	0	0	0	0	0	0	0	0
Karendi	Kw Kamau	0	0	0	0	9	11	5	10	0	4	6	7
	M Wanjuhi	0	0	0	0	0	0	0	0	0	0	0	0
Narua	M Wanjuhi	5	4	4	6	0	5	8	3	0	6	4,5	0

Appendix 8 (cont)

ANIMAL	FARMER	VM1	VM2	VM3	VM4	VM5	VM6	VM7	VM8	VM9	VM10	VM11	VM12
	M Wanjuki	0	0	0	0	0	0	0	0	0	0	0	0
Susan	Mw Kimari	2	3	3,5	4	4,5	2,5	0	0	0	0	0	7
Jane	Njoroge	0	0	0	0	0	0	0	0	0	0	0	0
Mathaga	Njoroge	0	0	0	0	0	0	0	0	0	0	0	0
Limura	P Nyacira	2	0	0	0	0	0	0	0	0	0	0	0
Naronja	P Nyacira	3	2,5	0	0	0	0	0	0	0	0	0	0
Muthiga	Pw Regild	2	4	5,5	3	0	0	0	2,1	1	0	0	0
Banana	Pw Regild	8	10	9	0	0	0	0	0	0	0	0	0
Kairitu	Pw Regild	0	5	6	5	0	0	0	0	5	0	0	7
Beauty	Pw Regild	5	5	6	6	0	0	0	7	5	0	0	7
	R Wanjiru	0	0	0	0	0	0	0	0	0	0	0	0
Summerset	Rw Chege	14	15	9,8	14	8	10	0	0	12	8	9	5
Kamau	Rw Chege	19	4	0	0	0	15	10	10	10	15	10	5
Kamori	Rw Chege	18	16	15	15	6	8	8	4	4	0	0	0
Kericho	Rw Chege	25	10	18,1	19	10	10	10	8	10	10	5	0
Mammrebu	Rw Chege	21	16	15	0	0	0	0	0	0	0	0	0
Mahatha	Rw Chege	23	20	17	15	12	10	8	6	0	10	9	10
Nyara	Rw Chege	5	2	5	0	0	0	0	0	0	0	0	0
Makiou	Rw Chege	10	2	0	2	1	0	0	0	0	0	0	0
Murembo	Rw Chege	17	16	15	15	8	8	6	5,6	0	10	9	10
Gilgil	Sn Mukuria	0	4	0	1	0	0	0	0	0	0	0	0
Kanini	Sn Mukuria	0	5	0	0	0	0	0	0	0	0	0	0
Matatha	Sn Mukuria	4	0	0	0	0	0	0	0	0	0	8	4,5
Mbarathi	Sn Mukuria	0	6	4	1,5	2	2,5	0	0	0	0	0	0
Munyaka	Sn Mukuria	3	7	4	1,5	1	0	0	0	0	0	0	0
Wanjiku	Sn Mukuria	0	8	4	1,5	3,5	2,5	1	1,4	0	0	12	9
Wanjiru	Sn Mukuria	0	5	3	1,5	2	1,5	2	1,4	4,5	4	6	5
Maridadi	Sn Mukuria	0	0	0	0	0	7	3	3,5	4,5	5	5	6
January	V Thidau	0	0	0	0	10	9	7,5	7	8	7	9	8
June	V Thidau	6	0	4	4	2,5	3,5	2,5	0	0	0	0	5
Juma	Vg Gitau	5	3	3	1,5	0	0	0	0	0	0	0	0
June	Vg Gitau	0	0	9	8	5,5	8,3	4,9	4,5	4,5	2,8	0	2,8
Monday	Vg Gitau	7,3	5	5	4	1,5	0	20	10	9	6,5	8	4
	W Mwaniki	0	0	0	0	0	0	0	0	0	0	0	0
June	W Mwaniki	0	0	0	0	0	0	0	0	0	0	6,5	12
Friday	W Mwaniki	8	7	7,5	4	7,5	0	0	0	0	0	0	0
Sunday	W Mwaniki	0	0	0	0	0	0	0	0	0	12	4,5	12
Mairo	Wairimu	6	3,5	0	0	9	8,5	9	8,4	7,4	4,5	9	9
	Wairimu	0	0	0	0	0	0	0	0	0	0	0	0
Ndikiri	Wairimu	7	5,5	8	4,5	1	1	0	0	13,3	6	12	12
	Wm Kimari	0	0	0	0	0	0	0	0	0	0	0	0
Sigona	Ek Kihoro	7	3,5	7	4	5	7	8	6	6	6	7	4
Kairitu	Ek Kihoro	0	0	15	14	14	14	15	8	8	8	9	4
Margaret	G Kanduma	0	11	5,5	4	6	5	3	5	4,5	4	3	8
July	G Kanduma	1	0	5,5	5	6	6	3	4	5	2	5	5,5
Meni	G Kanduma	0	0	8,5	5	4	4	3,5	5	4	4	4	5

Appendix 8 (cont)

ANIMAL	FARMER	VM1	VM2	VM3	VM4	VM5	VM6	VM7	VM8	VM9	VM10	VM11	VM12
Munge	G Kanduma	0	5	4	3	5	3	0	0	0	6	3	9
Ng'ethe	Jn Kimani	0	9	12	10	9	6,5	9	4	3	1,4	0,5	0
Kanyua	K Kamau	1	1	0	0	0	0	9	3	7	3,2	0	2,5
	K Karanja	0	0	0	0	0	0	0	0	0	0	0	0
Jane	K Muariama	5	5	4,5	3	4	5	4,5	5	2	2	5	4
Kanini	K Muariama	0	0	0	0	0	15	9	7	3	7	8	6
Nyameni	K Muariama	2	4,5	3,5	0	4	0	0	0	0	3	6	5
Gitihai	K Mwangi	6	4	3,5	5	6	3	5	6	0	0	5	10
Ngunu	K Mwangi	0	0	0	0	0	0	4	6	0	9	4	6
Rachel	K Mwangi	3	5	2	2	0	0	0	0	0	10	9	8
Munge	K Mwangi	5	11	9	6	6	4	6	6	4	5	6	0
Gitaru	K Mwangi	5	7	5	5	7	4	5	5	3	6	0	0
Findu	M Gitundu	0	8	13	12	4,5	11	9	7,5	4	8	2	5
Tunda	M Gitundu	8	10	6	8	6	6	3,5	9	3	1	3	4
Mathaga	M Gitundu	4	6	8	8	4	7,5	7	5	0	0	0	0
Njata	M Kagari	8	8	8	4,5	2,5	0	0	0	0	0	0	0
Bahati	M Kimani	7	4	6,5	5,5	3,5	4	2	5	1,5	4,5	1	2
Mathaga	M Kimani	7	4	5	4,5	3,5	4	0,5	3,5	1,5	0,5	0,5	2
Jane	M Ngugi	2	3	3	0,3	2	3	1	0	0	0	0	0
Susie	M Ngugi	2	1	5,5	1	3	0,5	0	7,5	0	0	0	0
Kinjo	Mn Gichana	9	7	6	6	6	6	4	1	0	0	13	18
Cucu	Mn Gichana	9	3,5	6	6	6	7	4	3	2	2,5	0	0
Wachuka	N Kabuchi	4	6	5	4	0	7	4	4	2,5	1	0	0
Nyaguthii	N Kabuchi	4	7	4	4	0	7	4	4	1,5	1,5	0	0
Findu	N Kamau	4	0	0	0	8	8	0	0	0	0	0	0
Jane	N Kamau	8	9,5	9	4	5	5	0	0	0	0	0	0
Margaret	N Mundati	5	0	5,5	0	0	0	0	0	0	0	0	8
Muthungu	N Mundati	0	4,9	0	4	0	3	4	4	1	0	0	0
Kameni	N Mundati	7	5	5,5	4	7	4	4	1	0	0	0	12
Kimori	VmGichanga	6	5	3	0	0	0	0	0	14	9	9	8
Dina	C Mburu	7	0	0	0	4	5	3	6	1,5	2	2,5	4
Lucy	C Mburu	8	6	4	4	2	3	3	4	2	2	2,5	4
Kahungura	C Mburu	6	4,5	4	3	2,5	2	2	4	1,5	1	0	2
Mwenge	C Mburu	6	5	4	4	3	3	2,5	4	2	1,5	2,5	2
Molo	C Mburu	5	4	4	3,5	0	2	2	4	1	1,5	2	1,5
Chania	C Mburu	0	0	0	0	0	0	0	0	0	0	0	5
Florida2	G Gathuru	5	5	4	1,5	1,5	5	3,5	3	0,5	0	0	0
	G Kagunda	0	0	0	0	0	0	0	0	0	0	0	0
	G Kagunda	0	0	0	0	0	0	0	0	0	0	0	0
Jane	G Kagunda	11	9	7	7	4	6	0	0	0	0	0	0
Kairitu	G Kagunda	8	0	0	12	11	9	18	8	12,5	10	10	7
	H Ngugi	0	0	0	0	0	0	0	0	0	0	0	0
Jack	H Ngugi	0	0	0	0	0	0	0	8	13	7	8	5
Kairitu1	H Ngugi	3	3,5	2,5	0	0	0	8	5	8	7	8	3
Kairitu2	H Ngugi	8	5	3	3	0	0	0	8	12	8	10	6
Kairitu3	H Ngugi	5	11	8	6	5	1,5	3	1,5	0	0	0	0

Appendix 8 (cont)

ANIMAL	FARMER	VM1	VM2	VM3	VM4	VM5	VM6	VM7	VM8	VM9	VM10	VM11	VM12
Kamitu6	H Ngugi	0	0	8	7	8	3	3	5	5,5	0	6	1
	I Mwaniki	0	0	0	0	0	0	0	0	0	0	0	0
Munge	I Mwaniki	0	0	0	0	0	12	4,5	3	0	6	4	0
Mary	I Mwaniki	4	4	3	3	2,5	2,8	3	2	0	0	0	0
Findu	I Mwaniki	5	6	5	4	4	5	0	0	0	11	6	0
Ciru	I Mwaniki	4	4	2	3	3	6,7	4	2	0	3	2	0
Tharuni	Im Njuguna	0	12	13	10	9	9	12	0	10	12	5	9
February	J Muhia	3	2	5	2,5	2	2,1	2,5	3	1,5	2	0,5	0
June	J Muhia	5	5	3	2,5	4	3	3	5	1,5	0	0	6
Kianduri	Jn Njoroge	6	5	5,5	5,5	6	4,5	2	3	2	2,1	5,5	3
Wakuhi	N Kaigwa	4	2,5	0	0	0	0	7,5	4,5	0	3	5	4
Kanini	P Njenga	0	6	0	0	0	0	0	0	0	7	10	7
Njata	P Njenga	8	0	7	5	5	6	5	4	3	1	0	9
Molo	Pk Giathi	8	7	6	5	2	1,5	0	5,6	10	5	7	0
Wanjiru	Pk Giathi	0	0	10	8	5	5	7	3,5	4	3,5	4	5
Wanyoko	Pk Giathi	7	7	6	5	2	1,5	0	0	10	6	9	7
Nyameni	Pm Gichua	5	5	0	7,5	8	0	6	5	5	3	4	4
Sunday	W Kimemia	4	4	1,5	1,5	1,5	0	0	0	0	0	0	0
Jane	W Njoroge	6	3,5	3,5	6	5	8	6	5	4,5	6	6	5
Mathaga	W Njoroge	5	3,5	3,5	3,5	3,5	9	1,5	3	0	0	0	12
Kandogo	Aw Micwe	0	5	3,5	2,1	0,5	3	1,5	0	2	0,5	0	0
Kahawa2	Aw Mwai	12	6	5	5	3,5	6	4	0	5	1	0	0
Ngamu	Gk Gaturu	7	7	3,5	0	3	4,5	3	4	4	0	0	0
Ndama	Gk Gaturu	7	4,2	0	0	0	6	8	12	10	2	8	10
	Gm Mugane	0	0	0	0	0	0	0	0	0	0	0	0
	Gm Mugane	0	0	0	0	0	0	0	0	0	0	0	0
Mwitindia	Gm Mugane	12	6	3,5	3,5	7	1	1,5	1,5	0	0	0	0
Mwogi	Gm Mugane	0	7	7	0	7	3	4	6,5	0	0	0	0
	Gw Ngugi	0	0	0	0	0	0	0	0	0	0	0	0
Kairitu	Gw Ngugi	4	4	0	0	4	10	12	9	8	3,5	10	7
Cucu	Gw Ngugi	4	0	5,5	0	0	0	12	9	9	5,5	10	7
Rose	Jc Nganga	4	0	3	0	0	0	0	0	0	0	0	0
Karendi	Jc Nganga	0	0	0	0	0	1	0	0	0	0	0	0
Kandogo	Lm Muigai	6	4,5	4,5	0	5	2	5	2,5	3,5	1	3	0
	Lm Muigai	0	0	0	0	0	0	0	0	0	0	0	0
Nyameni	Lm Muigai	4	6,5	5,5	10	0	6	10	2,5	5	4	8	6
Kajiado	Lm Muigai	0	0	0	6	7	3	7	5	6,5	3	6	9
Mine	Lw Waweru	11	0	9	7	5	5	0	0	14	10,5	12	15
Kariru	Lw Waweru	0	0	0	0	0	0	0	10	10	8,5	6,5	14
	Lw Waweru	0	0	0	0	0	0	0	0	0	0	0	0
Kanini	Mn Ndungu	3	4	3,5	2,5	1,5	1	1	0	0	0	11	10
Nyakairu	Mn Ndungu	6	5	7	3,5	2,5	3,5	2,5	5	4	3	4	3,5
Ester	Mn Kuria	6	1,5	5	5,5	0	0	0	4	5,5	4,5	5,5	7
Nyameni	Mt Kibiro	0	4	10	4	7	4	4	5	3,5	5,5	5,5	4,5
Marion	Mt Kibiro	4	4	4	2	0	0	7,5	7	0	7	6,5	5
	Mw Gatimu	0	0	0	0	0	0	0	0	0	0	0	0

Appendix 8 (cont)

ANIMAL	FARMER	VM1	VM2	VM3	VM4	VM5	VM6	VM7	VM8	VM9	VM10	VM11	VM12
	Mw Gatimu	0	0	0	0	0	0	0	0	0	0	0	0
Jane	Mw Gatimu	8	8,5	8,5	6	8,5	6	4	4,5	3	0	0	7
	Mw Gatimu	0	0	0	0	0	0	0	0	0	0	0	0
June	Mw Gatonye	4	5	3,5	5	4	3	3,5	2	3	2,8	2	3,5
	Ndirangu	0	0	0	0	0	0	0	0	0	0	0	0
13	Sn Githu	7	7	1,5	3	3	2	1,5	0	0	0	0	6
11	Sn Githu	5	5	2	2	3	2	2	2	7	2	0	3
14	Sn Githu	0	0	0	6	4	2	2	5,5	3	1	0	3
6	Sn Githu	0	0	0	4	4	2	2	3,5	5	1	0	3
8	Sn Githu	8	8	2	2	3	0	0	0	0	2	0	0
2	Sn Githu	0	0	0	0	0	0	0	0	0	0	0	0
3	Sn Githu	0	0	0	0	0	0	0	0	0	0	0	0
10	Sn Githu	0	0	0	0	0	0	0	0	4	2	0	2
1	Sn Githu	0	0	0	0	4	2	3	5	3	0,5	0	2,5
4	Sn Githu	4	4	1,5	0	2	0	2	0	0	0	0	0
7	Sn Githu	4	0	0	11	4	2	2	2	4,5	2	0	4
Kairu	Vn Kamau	7	1	0	6	10	12	6	5	4	0	7,5	4

Key

VM1...12 = milk production on visit 1...12 in l/day /cow

Appendix 9. Bodyweight, organ weight, fluoride levels in tissues of wistar rats

Case	Rat	Dose	Bwt	Days	Tissue	Kidney	Liver	Lungs	Heart	Dwt
1	3	1	301.4	196.0	1.000	M	M	M	M	4.000
2	1	1	322.6	253.0	1.000	2.590	15.99	19.22	17.70	15.60
3	1	1	322.6	253.0	1.000	M	M	M	M	15.60
4	2	1	321.8	606.0	1.000	M	M	M	M	9.100
5	6	1	343.0	843.0	1.000	2.600	17.10	20.84	18.98	12.40
6	6	1	343.0	843.0	1.000	M	M	M	M	11.44
7	4	1	270.9	96.00	2.000	2.210	11.64	14.64	13.00	532.2
8	3	1	301.4	196.0	2.000	M	M	M	M	597.0
9	3	1	301.4	196.0	2.000	M	M	M	M	427.6
10	7	1	303.7	197.0	2.000	M	M	M	M	389.7
11	2	1	321.8	606.0	2.000	M	M	M	M	571.3
12	2	1	321.8	606.0	2.000	M	M	M	M	414.8
13	7	1	303.7	197.0	3.000	2.300	15.60	18.86	17.15	579.3
14	1	1	322.6	253.0	3.000	M	M	M	M	308.2
15	4	1	270.9	96.00	4.000	M	M	M	M	344.5
16	5	1	245.5	96.00	4.000	1.940	10.48	13.10	11.54	234.6
17	3	1	301.4	196.0	4.000	M	M	M	M	402.7
18	1	1	322.6	253.0	4.000	M	M	M	M	367.0
19	1	1	322.6	253.0	4.000	M	M	M	M	209.3
20	2	1	321.8	606.0	4.000	M	M	M	M	386.5
21	3	2	322.4	209.0	1.000	2.410	15.07	18.70	17.04	5.160
22	5	2	301.6	258.0	1.000	M	M	M	M	5.180
23	1	2	307.8	104.0	2.000	M	M	M	M	325.4
24	2	2	306.9	104.0	2.000	M	M	M	M	416.6
25	6	2	376.0	197.0	2.000	M	M	M	M	751.7
26	4	2	340.0	209.0	2.000	2.510	15.26	18.74	17.0	801.9
27	5	2	301.6	258.0	2.000	M	M	M	M	448.7
28	1	2	307.8	104.0	3.000	2.440	13.55	16.61	14.70	268.2
29	2	2	306.9	104.0	3.000	2.650	14.71	18.90	17.07	255.8
30	6	2	376.0	197.0	3.000	M	M	M	M	402.1
31	7	2	310.6	206.0	3.000	M	M	M	M	219.8
32	5	2	301.6	258.0	3.000	M	M	M	M	491.9
33	1	2	307.8	104.0	4.000	M	M	M	M	168.4
34	2	2	306.9	104.0	4.000	M	M	M	M	308.0
35	6	2	376.0	197.0	4.000	M	M	M	M	271.0

Appendix 9 (cont)

Case	Rat	Dose	Bwt	Days	Tissue	Kidney	Liver	Lungs	Heart	Dwt
36	3	2	322.4	209.0	4.000	M	M	M	M	230.8
37	5	2	301.6	258.0	4.000	2.540	11.69	15.10	13.21	242.4
38	4	3	332.0	106.0	1.000	M	M	M	M	2.000
39	1	3	384.8	254.0	1.000	M	M	M	M	5.600
40	6	3	358.0	481.0	1.000	M	M	M	M	4.400
41	7	3	374.0	579.0	1.000	M	M	M	M	5.300
42	4	3	332.0	106.0	2.000	M	M	M	M	347.6
43	5	3	251.5	106.0	2.000	M	M	M	M	413.0
44	2	3	329.3	197.0	2.000	M	M	M	M	728.2
45	3	3	365.2	197.0	2.000	M	M	M	M	602.8
46	1	3	384.8	254.0	2.000	2.950	16.65	20.62	18.60	407.7
47	2	3	329.3	197.0	3.000	2.390	13.58	17.13	15.30	663.5
48	3	3	365.2	197.0	3.000	3.580	18.78	24.08	20.66	586.5
49	1	3	384.8	254.0	3.000	M	M	M	M	527.0
50	4	3	332.0	106.0	4.000	2.780	15.19	18.44	16.59	304.6
51	5	3	251.5	106.0	4.000	2.420	13.89	16.75	15.35	304.6
52	2	3	329.3	197.0	4.000	M	M	M	M	329.4
53	3	3	365.2	197.0	4.000	M	M	M	M	303.9
54	7	3	374.0	579.0	4.000	M	M	M	M	645.4
55	2	4	270.4	106.0	1.000	M	M	M	M	2.700
56	6	4	273.1	109.0	1.000	M	M	M	M	2.300
57	3	4	287.5	195.0	1.000	M	M	M	M	15.20
58	4	4	295.7	195.0	1.000	M	M	M	M	18.80
59	5	4	289.1	258.0	1.000	M	M	M	M	5.200
60	7	4	290.7	388.0	1.000	M	M	M	M	1.700
61	8	4	311.3	390.0	1.000	M	M	M	M	5.100
62	1	4	275.7	106.0	2.000	1.950	10.61	13.88	12.14	388.8
63	1	4	275.7	106.0	2.000	M	M	M	M	466.4
64	6	4	273.1	109.0	2.000	M	M	M	M	757.4
65	3	4	287.5	195.0	2.000	M	M	M	M	695.6
66	4	4	295.7	195.0	2.000	M	M	M	M	1124.3
67	5	4	289.1	258.0	2.000	M	M	M	M	1195.0
68	1	4	275.7	106.0	3.000	M	M	M	M	640.5
69	2	4	275.7	106.0	3.000	M	M	M	M	686.3
70	3	4	287.5	195.0	3.000	M	M	M	M	626.2

Appendix 9 (cont)

Case	Rat	Dose	Bwt	Days	Tissue	Kidney	Liver	Lungs	Heart	Dwt
71	4	4	295.7	195.0	3.000	M	M	M	M	1163.6
72	5	4	289.1	258.0	3.000	M	M	M	M	771.3
73	7	4	290.7	388.0	3.000	M	M	M	M	222.3
74	8	4	311.3	390.0	3.000	M	M	M	M	1781.0
75	1	4	275.7	106.0	4.000	M	M	M	M	400.5
76	2	4	270.4	106.0	4.000	M	M	M	M	449.8
77	3	4	287.5	195.0	4.000	M	M	M	M	252.3
78	4	4	295.7	195.0	4.000	2.310	15.03	18.04	16.39	670.1
79	5	4	289.1	258.0	4.000	2.270	12.11	15.08	13.61	597.8
80	7	4	290.7	388.0	4.000	M	M	M	M	1703.8
81	8	4	311.3	390.0	4.000	M	M	M	M	1896.9
82	3	5	306.6	195.0	1.000	M	M	M	M	11.70
83	4	5	297.6	195.0	1.000	2.540	14.78	18.25	16.48	13.40
84	6	5	269.0	347.0	1.000	M	M	M	M	8.500
85	6	5	269.0	347.0	1.000	M	M	M	M	8.000
86	6	5	269.0	347.0	1.000	M	M	M	M	96.70
87	1	5	321.7	107.0	2.000	2.490	13.55	16.41	14.72	1224.3
88	2	5	250.4	107.0	2.000	2.130	13.13	15.61	14.31	1575.7
89	3	5	306.6	195.0	2.000	2.370	12.55	16.25	13.92	1951.1
90	7	5	290.4	197.0	2.000	M	M	M	M	835.2
91	6	5	269.0	347.0	2.000	M	M	M	M	1598.0
92	6	5	269.0	347.0	2.000	M	M	M	M	3949.2
93	6	5	269.0	347.0	2.000	M	M	M	M	2809.6
94	5	5	300.2	258.0	2.000	2.330	13.36	16.30	14.72	2023.2
95	1	5	321.7	107.0	3.000	M	M	M	M	1716.2
96	2	5	250.4	107.0	3.000	M	M	M	M	1126.0
97	3	5	306.6	195.0	3.000	M	M	M	M	1201.5
98	7	5	270.4	197.0	3.000	2.100	9.900	12.78	10.85	503.2
99	6	5	269.0	347.0	3.000	M	M	M	M	249.6
100	6	5	269.0	347.0	3.000	M	M	M	M	6.000
101	6	5	269.0	347.0	3.000	M	M	M	M	543.8
102	1	5	321.7	107.0	4.000	M	M	M	M	1124.8
103	2	5	250.4	107.0	4.000	M	M	M	M	1285.2
104	3	5	306.5	195.0	4.000	M	M	M	M	911.4
105	4	5	297.6	195.0	4.000	M	M	M	M	713.0

Appendix 9 (cont)

Case	Rat	Dose	Bwt	Days	Tissue	Kidney	Liver	Lungs	Heart	Dwt
106	5	5	300.2	258.0	4.000	M	M	M	M	431.0
107	6	5	269.0	347.0	4.000	M	M	M	M	943.0
108	6	5	269.0	347.0	4.000	M	M	M	M	1440.7
109	6	5	269.0	347.0	4.000	M	M	M	M	1286.6
110	3	6	299.1	166.0	1.000	M	M	M	M	14.00
111	7	6	287.0	342.0	1.000	2.350	12.94	17.01	15.25	21.50
112	7	6	287.0	342.0	1.000	M	M	M	M	62.00
113	7	6	287.0	342.0	1.000	M	M	M	M	22.30
114	7	6	287.0	342.0	1.000	M	M	M	M	54.30
115	1	6	300.2	107.0	2.000	2.940	14.31	17.08	15.53	1859.9
116	2	6	288.1	107.0	2.000	2.140	13.68	17.38	15.30	1789.1
117	3	6	299.1	166.0	2.000	2.180	12.84	15.83	14.24	2223.2
118	4	6	307.1	166.0	2.000	2.590	15.54	18.46	16.78	2244.7
119	5	6	287.0	253.0	2.000	1.910	9.950	12.85	11.12	2411.5
120	6	6	295.1	335.0	2.000	M	M	M	M	2517.1
121	6	6	295.1	335.0	2.000	M	M	M	M	2287.7
122	7	6	287.0	342.0	2.000	M	M	M	M	2758.9
123	7	6	287.0	342.0	2.000	M	M	M	M	3039.0
124	1	6	300.2	107.0	3.000	M	M	M	M	1230.9
125	3	6	299.1	166.0	3.000	M	M	M	M	1601.7
126	4	6	307.1	166.0	3.000	M	M	M	M	1981.2
127	5	6	287.0	253.0	3.000	M	M	M	M	1773.7
128	7	6	287.0	342.0	3.000	M	M	M	M	1853.8
129	7	6	287.0	342.0	3.000	M	M	M	M	2502.7
130	7	6	287.0	342.0	3.000	M	M	M	M	2071.0
131	7	6	287.0	342.0	3.000	M	M	M	M	1619.0
132	1	6	300.2	107.0	4.000	M	M	M	M	1166.2
133	2	6	288.1	107.0	4.000	M	M	M	M	964.8
134	3	6	299.1	166.0	4.000	M	M	M	M	1892.4
135	4	6	307.1	166.0	4.000	M	M	M	M	2107.6
136	5	6	287.0	253.0	4.000	M	M	M	M	1361.0
137	7	6	292.5	340.0	4.000	M	M	M	M	1161.9
138	7	6	292.5	340.0	4.000	M	M	M	M	2535.1
139	7	6	287.0	342.0	4.000	M	M	M	M	1560.8
140	7	6	287.0	342.0	4.000	M	M	M	M	1618.8
141	2	7	166.6	126.0	1.000	1.78	8.400	10.76	9.45	89.00

Appendix 9 (cont)

Case	Rat	Dose	Bwt	Days	Tissue	Kidney	Liver	Lungs	Heart	Dwt
142	4	7	197.9	206.0	1.000	M	M	M	M	3.700
143	5	7	165.1	218.0	1.000	M	M	M	M	9.100
144	6	7	195.5	218.0	1.000	M	M	M	M	10.20
145	7	7	177.1	253.0	1.000	M	M	M	M	25.70
146	8	7	176.0	606.0	1.000	M	M	M	M	5.000
147	5	7	165.1	218.0	2.000	M	M	M	M	1287.0
148	6	7	195.5	218.0	2.000	M	M	M	M	907.5
149	3	7	230.5	166.0	2.000	M	M	M	M	792.4
150	4	7	197.9	166.0	2.000	M	M	M	M	935.2
151	4	7	197.9	206.0	2.000	M	M	M	M	309.9
152	7	7	177.1	253.0	2.000	M	M	M	M	845.2
153	9	7	183.7	441.0	2.000	M	M	M	M	565.6
154	9	7	183.7	441.0	2.000	M	M	M	M	590.3
155	8	7	176.0	606.0	2.000	M	M	M	M	1292.9
156	1	7	203.6	104.0	3.000	M	M	M	M	379.0
157	2	7	166.6	126.0	3.000	M	M	M	M	302.6
158	3	7	230.5	166.0	3.000	M	M	M	M	563.7
159	4	7	197.9	166.0	3.000	M	M	M	M	494.1
160	7	7	177.1	253.0	3.000	M	M	M	M	794.6
161	10	7	168.7	380.0	3.000	M	M	M	M	799.6
162	10	7	183.7	380.0	3.000	M	M	M	M	253.7
163	8	7	176.0	606.0	3.000	M	M	M	M	1467.2
164	4	7	197.9	206.0	3.000	M	M	M	M	529.8
165	2	7	166.6	126.0	4.000	M	M	M	M	367.3
166	2	7	166.6	126.0	4.000	M	M	M	M	365.9
167	3	7	230.5	166.0	4.000	2.170	10.58	13.24	11.47	50.00
168	4	7	197.9	166.0	4.000	1.870	9.100	11.65	10.29	990.9
169	4	7	197.9	206.0	4.000	M	M	M	M	540.2
170	5	7	165.1	218.0	4.000	2.230	8.340	10.88	9.250	632.3
171	6	7	195.5	218.0	4.000	2.080	10.45	12.46	11.14	843.7
172	7	7	177.1	253.0	4.000	M	M	M	M	813.4
173	8	7	176.0	606.0	4.000	M	M	M	M	919.6
174	1	8	256.5	163.0	1.000	1.900	10.60	13.26	11.64	11.00
175	2	8	283.7	163.0	1.000	2.120	13.89	17.16	15.14	23.80
176	3	8	296.9	258.0	1.000	M	M	M	M	5.200

Appendix 9 (cont)

Case	Rat	Dose	Bwt	Days	Tissue	Kidney	Liver	Lungs	Heart	Dwt
177	1	8	256.5	163.0	2.000	M	M	M	M	640.2
178	2	8	283.7	163.0	2.000	M	M	M	M	639.5
179	3	8	296.9	258.0	2.000	M	M	M	M	376.4
180	4	8	290.9	126.0	3.000	M	M	M	M	270.4
181	1	8	256.5	163.0	3.000	M	M	M	M	379.4
182	2	8	283.7	163.0	3.000	M	M	M	M	512.4
183	3	8	296.9	258.0	3.000	M	M	M	M	388.2
184	1	8	256.5	163.0	4.000	M	M	M	M	336.9
185	2	8	283.7	163.0	4.000	M	M	M	M	424.6
186	3	8	296.9	258.0	4.000	M	M	M	M	232.2
187	1	9	118.8	127.0	1.000	M	M	M	M	122.6
188	2	9	143.1	127.0	1.000	M	M	M	M	4.600
189	3	9	179.3	163.0	1.000	M	M	M	M	14.50
190	3	9	179.3	163.0	1.000	M	M	M	M	7.800
191	5	9	175.4	254.0	1.000	M	M	M	M	8.200
192	5	9	175.4	254.0	1.000	M	M	M	M	4.900
193	4	9	177.6	340.0	1.000	M	M	M	M	1.200
194	6	9	175.0	342.0	1.000	M	M	M	M	26.40
195	7	9	170.0	352.0	1.000	M	M	M	M	1.000
196	8	9	210.0	390.0	1.000	M	M	M	M	6.500
197	1	9	118.8	127.0	2.000	M	M	M	M	430.1
198	2	9	143.1	127.0	2.000	M	M	M	M	492.3
199	3	9	179.3	163.0	2.000	M	M	M	M	975.3
200	3	9	179.3	163.0	2.000	M	M	M	M	928.1
201	5	9	175.4	254.0	2.000	M	M	M	M	519.6
202	5	9	175.4	254.0	2.000	M	M	M	M	433.2
203	6	9	175.0	342.0	2.000	M	M	M	M	2554.4
204	7	9	170.0	352.0	2.000	M	M	M	M	604.3
205	7	9	170.0	352.0	2.000	M	M	M	M	475.5
206	8	9	210.0	390.0	2.000	2.660	13.38	15.83	14.47	860.9
207	1	9	118.8	127.0	3.000	M	M	M	M	366.0
208	2	9	143.1	127.0	3.000	M	M	M	M	243.2
209	3	9	179.3	163.0	3.000	M	M	M	M	344.1
210	3	9	179.3	163.0	3.000	M	M	M	M	272.3
211	5	9	175.4	254.0	3.000	M	M	M	M	474.9

Appendix 9 (cont)

Case	Rat	Dose	Bwt	Days	Tissue	Kidney	Liver	Lungs	Heart	Dwt
212	5	9	175.4	254.0	3.000	M	M	M	M	555.2
213	4	9	172.6	340.0	3.000	M	M	M	M	646.8
214	6	9	175.0	342.0	3.000	M	M	M	M	245.0
215	7	9	170.0	352.0	3.000	M	M	M	M	403.5
216	7	9	170.0	352.0	3.000	M	M	M	M	616.5
217	8	9	210.0	390.0	3.000	M	M	M	M	1290.6
218	1	9	118.8	127.0	4.000	1.630	5.900	7.840	6.720	504.7
219	2	9	143.1	127.0	4.000	1.910	7.720	9.650	8.560	453.4
220	3	9	179.3	163.0	4.000	2.310	9.420	11.78	10.38	559.6
221	3	9	179.3	163.0	4.000	M	M	M	M	436.5
222	5	9	175.4	254.0	4.000	2.220	12.70	15.64	13.91	241.0
223	5	9	175.4	254.0	4.000	M	M	M	M	573.3
224	4	9	172.6	340.0	4.000	M	M	M	M	806.7
225	6	9	175.0	342.0	4.000	M	M	M	M	389.9
226	7	9	170.0	352.0	4.000	M	M	M	M	406.5
227	7	9	170.0	352.0	4.000	M	M	M	M	434.9
228	8	9	210.0	390.0	4.000	M	M	M	M	585.4
229	2	10	251.5	127.0	1.000	M	M	M	M	107.1
230	3	10	305.4	160.0	1.000	M	M	M	M	7.100
231	2	10	251.5	127.0	2.000	M	M	M	M	479.8
232	2	10	251.5	127.0	2.000	M	M	M	M	450.8
233	3	10	305.4	160.0	2.000	M	M	M	M	428.9
234	4	10	300.4	160.0	2.000	M	M	M	M	409.0
235	2	10	251.5	127.0	3.000	M	M	M	M	273.1
236	2	10	251.5	127.0	3.000	M	M	M	M	327.7
237	3	10	305.4	160.0	3.000	2.540	14.11	17.23	15.42	234.7
238	4	10	300.4	160.0	3.000	M	M	M	M	225.2
239	2	10	251.5	127.0	4.000	2.370	11.27	14.34	12.74	442.9
240	2	10	251.5	127.0	4.000	M	M	M	M	226.9
241	3	10	305.4	160.0	4.000	M	M	M	M	174.6
242	4	10	300.4	160.0	4.000	2.320	13.64	16.93	15.25	215.0

Key: M = sample not collected

Appendix 10.Standard calibration curve data

CASE	F/ppm	RM1	RM2	RM3	RM4	RM5	RM6	RM7	RM8	RM9
1	0.02	-9	5	342	340	344	180	-50	173	-85
2	0.10	-20	-18	316	316	317	155	-58	152	-75
3	1.00	-73	-72	260	260	260	100	-109	97	-120
4	10.00	-131	-131	201	201	201	45	-166	31	-175

CASE	RM10	RM11	RM12	RM13	RM14	RM15	RM16	RM17	RM18	RM19
1	-23	151	122	135	133	-65	151	119	-10	-8
2	-62	138	99	95	97	-129	120	89	-30	-30
3	-116	83	42	38	36	-184	65	42	-80	-81
4	-176	22	18	-21	-21	-241	1	23	-150	-140

CASE	RM20	RM21	RM22	RM23	RM24	RM25	RM26	RM27	RM28
1	-19	-18	156	136	102	102	98	99	99
2	-35	-33	147	134	93	93	86	90	91
3	-84	-89	92	91	38	42	37	37	39
4	-145	-148	34	32	-21	-17	19	-21	-19

Key:

RM = Relative millivolt values