TITLE PAGE

DETERMINATION OF THIOCYANATE AMONG SMOKERS AND NON-SMOKERS USING URINE AS A BIOLOGICAL INDICATOR AND OTHER CONTRIBUTING FACTORS OF CYANIDE EXPOSURE

By

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DECLARATION

I hereby declare that this is my original work and has not been presented for the award of a degree in any other university.

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DEDICATION

This thesis is dedicated to Almighty God, my parents; Mr. and Mrs. Nyachoti, my brothers; Vincent and Dennis, my sisters; Leonida and Mercy and my niece; Susan.

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LIST OF ABBREVIATIONS AND ACRONYMS

ATSDR	Agency for Toxic Substances and Diseases Registry
ANOVA	Analysis of Variance
CBS	Central Bureau of Statistics
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
EDTA	Ethylenediaminetetraacetic Acid
IARC	International Agency for Research on Cancer
LOD	Limit of Detection
MS	Mainstream
MMAD	Mass Median Aerodynamic Diameter
metHb	Methemoglobin
NCAPD	National Coordination Agency for Population and Development
NIOSH	National Institute for Occupational Safety and Health
NIH	National Institute of Health
PCI	Percutaneous Coronary Intervention
SS	Sidestream
SPSS	Statistical Package for Social Sciences or Statigraph software
US EPA	United States Environmental Protection Agency
WHO	World Health Organization

ABSTRACT

The low levels of thiocyanate (SCN) normally present in body fluids increase with chronic exposure to cyanide. Cyanide (CN^-) is a fast-acting, potentially deadly chemical which prevents or inhibits cellular respiration and inactivation of cytochrome oxidase killing the cell. Humans are exposed to cyanide from dietary, industrial, environmental and other sources. Tobacco smoking is an important source of cyanide exposure. After absorption, cyanides are readily distributed in the body through the blood and are converted to thiocyanate by sulphur transferase enzyme. Thiocyanates are removed from the body through urine. Thus, the determination of urine SCN is a necessary study in establishing the extent of cyanide overload. This study was therefore set to evaluate the concentration of thiocyanate in urine of smokers (n = 128) and non-smokers (n = 123) aged between 20-70 yrs as a biomarker of cyanide exposure. The sampling covered Kahawa Wendani, Kahawa Sukari, Githurai, Ruiru, Kiwanja and Kenyatta University in Nairobi and Thika, Kenya. Information on factors that influence the accumulation of cyanide was assessed using a questionnaire. The UV-visible spectrometer was used to determine the concentration of the thiocyanate using the picrate paper method, developed by Bradbury et al. The mean thiocyanate measured in the urine of smokers was 3.89 ± 0.17 mg/L and that of non-smokers was 1.99 ± 0.12 mg/L. The mean SCN levels in smokers were significantly higher than those of non-smokers ($P<0.05$; df = 230). Factors that were found to have significant influence were diet particularly cassava and sorghum which gave values of 4.16±0.33 mg/L and 3.75±0.27 mg/L respectively. Utilization of cassava in Kenya is limited to a few places, however, cassava and its products have been reported to be highly used in western (Busia) and coastal regions of the country. The duration and number of cigarettes per day were found to have a statistically significant influence. Fifteen samples purposively sampled were analysed using Lundquist method mainly for comparison purposes. For the samples analysed by these methods, a regression line gave very good agreement ($r^2 = 0.9942$). This information can be used to sensitize the public on the effects of cigarette smoke. The results of this study can also be used by the government to formulate policies and strategies to reduce cyanide exposure thereby addressing some of the health issues affecting people in Kenya

CHAPTER ONE

INTRODUCTION

1.1 Background information on cyanide

Cyanide (CN^-) is generalized as a chemical compound that contains one atom of carbon triple bonded to one atom of nitrogen. A cyanide molecule is readily capable of bonding with another atom or compound. The type of atom or compound that the cyanide molecule bonds to determines the properties and toxicity of the resulting product. For example if the cyanide molecule bond to hydrogen, hydrogen cyanide (HCN) is formed which is more toxic than when alkyl group is attached for instance CH_3CN. Thiocyanates (SCN) are groups of cyanide with a sulphur molecule attached (ATSDR, 2004).

Cyanide is a fast acting, potentially deadly chemical which prevents or inhibits cellular respiration and inactivation of cytochrome oxidase hence death of body cells. However, minute amounts of cyanide in form of vitamin B_{12} (cyanocobalamine) are necessary requirement in human diet (Li *et al*., 2000). Compounds capable of releasing cyanide may be inorganic or organic in nature. Inorganic compounds may be simple (AgCN, KCN) or complex ($KAg(CN)_2$). Organic compounds may be glycosidic or nitriles found in consumable foods like cassava, fruits, and cereals such as sorghum and millet. Free cyanides include cyanides present as either HCN or CN^- or SCN^- (Gosselin *et al*., 1984).

Cyanide of all form is poisonous. These include cyanide salts either in solid, liquids and the gas, HCN (also known as hydrocyanic acid or prussic acid). Cyanide exposure in man

may arise from both natural and anthropogenic sources as well as produced by certain bacteria, fungi and algae (Li *et al*., 2000).

Higher concentrations of cyanide in the body may be due to inhalation of tobacco or fire smoke, use of certain drugs such as sodium nitroprusside, automobile exhausts, chemical manufacturing industries, volatilization from cyanide wastes, combustion of synthetic polymers and fumigation operations. Humans get exposed to cyanide mainly by breathing air, drinking water, eating food or touching soils. Too much of cyanide in body is characterized by headache, nausea, rapid breathing and vomiting. Chronic exposure may lead to paralysis of legs, a disease called Konzo and even death (Banea *et al*., 1992). There are several disorders which have been associated with regular intake of sub-lethal quantities of cyanogenic glucosides. These include goiter and cretinism (Delenge *et al*., 1994) and tropical ataxic neuropathy (Osuntokun, 1994).

1.2 Anthropogenic and natural sources of cyanide

Anthropogenic (man-made) sources of cyanide may include synthetic catalytic processes involving reaction of ammonia and natural gas with or without air. Hydrogen cyanide may be obtained as a byproduct in the production of acrylonitrile. Other cyanides like sodium and potassium cyanides are principally prepared by direct reaction of hydrogen cyanides released to air as a result of chemical manufacturing and processing industries, volatilization from cyanide wastes deposited in land fills and waste ponds (Carotti and Kaiser, 1972). Hydrogen cyanide is present in vehicle exhausts and tobacco smoke.

Earlier studies showed that smoke due to incomplete combustion of plastics and house fires often results in cyanide poisoning (Carotti and Kaiser, 1972).

Naturally, cyanide is released to the environment from biomass burning, volcanoes and natural biogenic processes from higher plants, bacteria, algae and fungi (Li *et al*., 2000). Cyanide also occurs naturally as cyanogenic glycosides in at least 2000 plants including fruits and vegetables (Padmaja, 1995). Examples include cassava, sweet potatoes, corn, cabbage, linseeds, millet and bamboo, in pits of stone fruits such as cherries, peaches, apricots and in apple seeds (Padmaja, 1995). It is also present in bitter almonds and American white lima beans (Ermans *et al*., 1972). Cyanogenic glycosides in plants include linamarin, lotaustralin, amygdalin, prunasin, Dhrurrin (as listed in Table 1.0). These are hydrolysed to release (CN^-) and d- glucose by enzymes as shown in Equation 1.0 (Frakes *et al*., 1986a).

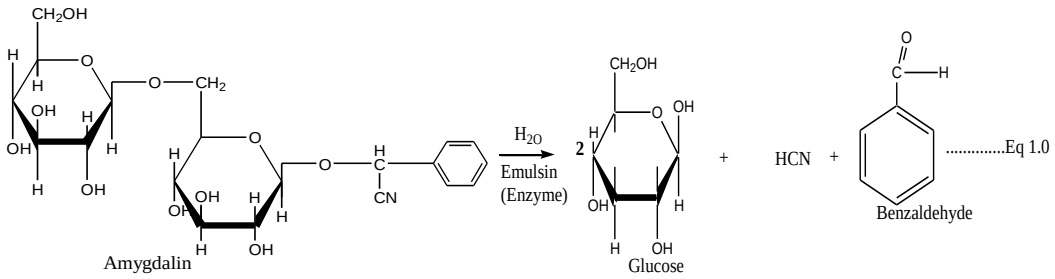
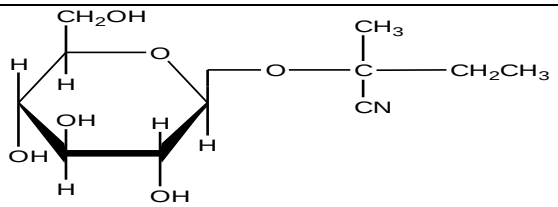
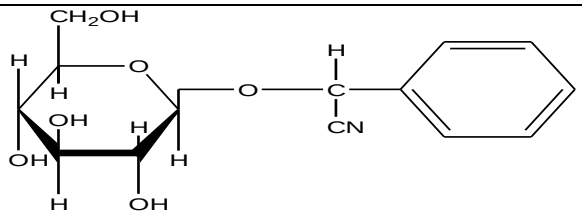
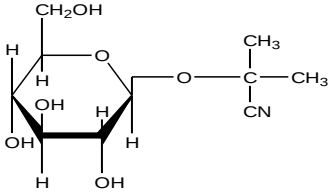
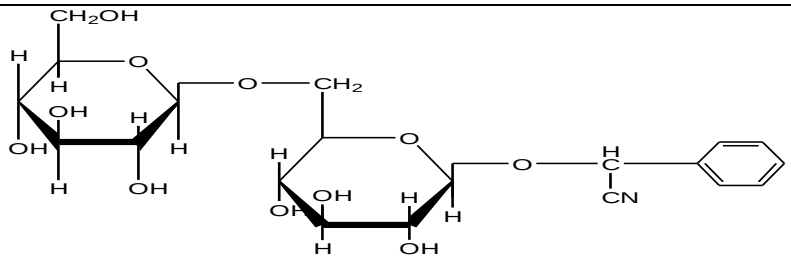
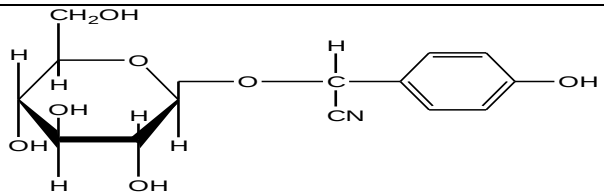


Table 1.0: The cyanogenic glycosides in plants

Type of cyanogenic glycoside	Structure	Plant found
Lataustralin		Cassava and lima beans
Prunasin		Stone fruits like apples, peaches and cherries.
Linamarin		Cassava
Amygdalin		Almonds
Dhrurrin		Sorghum

Source: Frakes *et al*., 1986a

Cassava (*Manihot esculenta*) is the staple food of more than 500 million people in the tropics (Cock, 1985). In Kenya cassava has limited utilization due to lack of knowledge and skills in processing. The knowledge concerning cyanide content also hampers its diverse utilization and market (Karuri *et al*., 2001). Cassava is one of the major sources of hydrogen cyanide and a major health concern as shown by studies carried out by Bradbury et al (1999). When the cellular structure of the cassava is disrupted, the intracellular glucoside becomes exposed to the extracellular enzyme like linamarase, hydrocyanic acid is produced. Linamarin is rapidly hydrolysed to glucose and acetone cyanohydrin catalysed by linamarase. Lotaustralin is also hydrolysed to a related cyanohydrin and glucose. Under neutral or alkaline conditions, acetone cyanohydrin decomposes to acetone and HCN/CN^- (Cooke *et al*., 1985). The high value of urine thiocyanate results from inhalation of HCN which readily passes the lungs into the blood stream and is converted to SCN in the liver and kidneys. Human body fluids like serum, plasma, saliva and urine have been used as biological indicators in the study of thiocyanate in the body (Lauwerys and Hoet, 2001). Determination of SCN compound in urine has been used to indicate cyanide overload (Bradbury *et al*., 1999).

The cyanogenic glycosides concentrations in many edible plants can vary widely as a result of genetic and environmental factors, location, seasons and soil types (Ermans *et al*., 1980). Some of the foodstuffs and their cyanide contents are shown in table 1.1 (Ermans *et al*., 1980).

Table 1.1: Cyanide concentrations in food products

Type of product	**Cyanide concentration(mg/L)**
Cereal grains and their products	0.001-0.45
Soy protein products	0.07-0.3
Soybean hulls	1.24
Apricot pits, wet weight	89-2170
Home-made cherry juice containing 100% crushed pits	23
Commercial fruit juices	
Cherry	4.6
Apricot	2.2
prune	1.9
Tropical foodstuffs	
Cassava (bitter)/dried root cortex	2360
Cassava (bitter)/ whole tubers	380
Cassava (sweet)/ leaves	451
Cassava (sweet)/ whole tubers	445
Gari flour (Nigeria)	10.6-22.1
Sorghum/whole immature plant	2400
Lima beans	2900

Source; Honig *et al*., 1983

1.3 Applications of cyanide

Cyanides have a wide range of applications. Inorganic cyanides are used in electroplating, extraction of gold and silver ores, metal processing, photographic processes, production of plastics, pesticides and rodenticides, dehairing of hides, laboratory processes and manufacturing of dyes and pigments (Ware, 1989). Organic cyanides of various types find wide applications and are of great commercial significance. They are used in industrial processes as solvents and as intermediates in synthesis and some find use as pesticides and as ingredients of fragrances. Besides this, organic cyanides, as cyanogenetic glycosides, occur in many plant materials, some of which are used as foodstuffs (Padmaja, 1995).

1.4 Tobacco smoking

A higher concentration of thiocyanate that is a metabolic product of cyanide arises from tobacco smoke (Michigami *et al*., 1992). Tobacco smoking is the act of burning dried or cured leaves of tobacco plant and inhaling the smoke for pleasure, for ritualistic or social purposes, self medication or simply to satisfy physical dependence or addiction. The cigarette is the most common method of smoking tobacco; others may include cigar and pipes smoking and crude tobacco sniffing (Doll and Hill, 1950).

1.4.1 Definition of cigarette

A cigarette is a product consumed through smoking and manufactured out of cured and finely cut tobacco leaves and reconstituted tobacco, combined with other additives then rolled or stuffed into a paper-wrapped cylinder (generally less than 120 mm in length and 10 mm in diameter) (Doll and Hill, 1950). The cigarette is ignited at one end and allowed to smoulder for the purpose of inhalation of its smoke from the other (usually filtered) end, which is inserted in the mouth. They are sometimes smoked with a holder. A cigarette is distinguished from a cigar by its smaller size, use of processed leaf, and white paper wrapping. Cigars are typically composed entirely of whole-leaf tobacco (Gillilang *et al*., 2006).

1.4.2 Combustion of a cigarette

During a puff, the periphery of the cigarette burns faster than the apex. The maximum temperature attainable at the periphery is about 900^{o}C when the cigarette is puffed. The recorded temperature in the centre of the coal (some 8-10 mm from the line of the paper burns) exceeds 800^{o}C (IARC, 1986). This coal temperature has a significant effect on

some composition, the higher the coal temperatures the more gaseous the smoke and the less particulate it is (Henningfield and Griffins, 1981).

The actual combustion of a single cigarette is dependent upon several factors. These include the width of tobacco cut, density of cigarette, moisture content of the tobacco, diameter of the cigarette, shape of the cigarette (round and oval), permeability of the paper used (increase in permeability results in decrease in coal temperature, filter dilution or ventilation (increase in dilution decreases coal temperatures) and puff volume (with higher volumes increasing coal temperature) (Henningfield and Griffins, 1981).

Cigarette smoke penetrates deep into the lungs and reaches the small airways and alveoli. The fraction of smoke deposited is high because most smokers employ some breath – holding following inhalation of a puff. Their attempt to enhance deposition of smoke results in increased lung burdens of toxic smoke products like hydrogen cyanide (Sherman, 1991). Non-smokers are also exposed to tobacco combustion products when other people smoke. These are termed as passive or involuntary smokers. Studies estimate that non-smokers exposed to passive smoke absorb an equivalent of 0.1-1.0 cigarette per day (Sherman, 1991). Urine SCN reference ranges for normal smoker and non-smoker have not been established but urinary concentrations of SCN have been reported to be similar to those in blood (Scherer, 2006). Considering blood SCN reference ranges, <0.02 mg/L for normal non-smoker and 0.041 mg/L for normal smoker have been reported. Exposure to levels of <0.2 mg/L have been found to be non-toxic while exposure to 10 mg/L has been associated with death (Ryan and Terry, 1997).

1.4.3 Cigarette smoke

Cigarette smoke is a complex matrix containing approximately 4000 different constituents separated into gaseous and particulate phases (Hoffman *et al*., 2001). The components of the gaseous phase include carbon monoxide, carbon dioxide, ammonia, nitrogen dioxide, hydrogen cyanide, volatile sulphur-containing compounds, nitrogen oxides (including nitric oxide, NO), and other nitrogen-containing compounds. The particulate phase contains nicotine, water and tar (Guerin, 1980).

1.4.4 Environmental tobacco smoke

Tobacco smoke is an aerosol consisting of a particulate phase of liquid droplets dispersed in a gas/vapour phase. The smoke in the environment is derived from three sources: Mainstream (MS), second-hand smoke (SHS) and sidestream (SS) (Hoffmann and Hoffmann, 1997). When a cigarette is smoked, many compounds are formed by pyrolysis of the tobacco. These either pass through the cigarette as MS smoke, some being condensed a short distance behind the burning cone, or they are emitted into the air from the burning end as SS smoke. With each puff the smoke becomes progressively stronger because previously condensed material is added to the smoke and the length of cigarette available for further condensation is decreasing. The physiochemical nature of the smoke depends on the processing and burning of the tobacco, the porosity and treatment of the paper wrapper, and on the type of filter tip (Hoffmann and Hoffmann, 1997). In the case of a cigarette or Asian "bidi" (tobacco wrapped in vegetable leaf), the smoke chemistry is affected by such factors as dimensions, wrapper porosity and the smoking parameters of puff volume, frequency and duration (NIH, 1998). Variations in smoke chemistry are

mainly in the balance of smoke constituents rather than the presence or absence of particular compounds.

Mainstream smoke is generated in a comparatively low-oxygen atmosphere at a burning temperature of 850-950°C in the fire cone. Initially, MS smoke particles have a mass median aerodynamic diameter (MMAD) of 0.2 to 0.3 µm; however, as soon as they encounter the 100 % humidity of the respiratory tract, they coalesce into larger particles and behave as if their MMAD was in the micrometre range. Between 50 and 90 % of all inhaled particulate matter may be retained in the respiratory tract (Hoffmann *et al*., 2001). From size considerations, the aerosol particulate matter, the vapour phase constituents and the permanent gases are capable of reaching the alveoli when smoke is inhaled. Deposition in the tracheobronchial tree is complicated by the behaviour of hydrophilic constituents in the high humidity conditions, but smoke reaches every part of the airways.

Mainstream smoke contains nearly 4000 identified chemicals and an unknown number of unidentified chemicals (Roberts, 1988). Mainstream smoke can be divided into particulate and gas phases. The vapour phase contains carbon monoxide, carbon dioxide, benzene, ammonia, formaldehyde, hydrogen cyanide, *N*-nitrosodimethylamine, *N*-nitrosodiethylamine and other compounds. Reported yields of HCN in MS smoke of cigarettes vary between 150 and 500 µg/cigarette. Earlier research by Artho and Koch, 1950 has reported a hydrogen cyanide level of 150-300 µg/cigarette (Baumeister *et al*., 1975).

Sidestream smoke is generated at lower burning temperature (500-600°C) in a reducing atmosphere. Fresh SS smoke particles are about the same size as mainstream smoke particles with a MMAD of approximately 0.2 µm qualitatively, sidestream smoke composition is similar to the composition of mainstream smoke. Some chemicals in SS are emitted at higher concentrations per gram of tobacco burned than in MS smoke. The HCN is formed in the burning zone mainly from proteins and nitrates at temperatures $>700^0$C oxygen deficient conditions. This is why HCN yields in sidestream smoke (lower combustion temperature, better oxygen supply) are lower than mainstream smoke (14-134 µg/cigarette) (IARC, 1986). Second-hand smoke is the combination of SS smoke (the smoke given off by the burning end of a tobacco product) and MS smoke (the smoke exhaled by the smoker). It has same chemical composition as SS or MS smoke. Approximately one non-smoker dies due to second-hand smoke exposure for every eight smokers dying of smoking related diseases (Schick and Glantz, 2006).

1.4.5 Health risks of tobacco smoking

The health risks of smoking are not uniform across all smokers. Risks vary according to amount of tobacco smoked, with those who smoke more at greater risk. Light smoking is still a health risk. The data regarding smoking to date focuses primarily on cigarette smoking, which even by conservative estimates increases mortality rates by 40%. Men who smoke 10-19 cigarettes a day have a 70% increase in mortality rates, men who smoke 20-39 cigarettes a day have an increase in mortality rate by 90%, while men smoking two packs a day or more, have their mortality rates increased to120% (Doll and Hill, 1950).

The health effects of tobacco smoking are related to direct or passive smoking, inhalation of environmental or second-hand tobacco smoke. A 50 year study of over thirty thousand British physicians showed that non-smokers live about 10 years longer than smokers (Doll *et al.*, 2004). The chemicals in tobacco smoke are considered toxic because they have serious health impacts on the human body. For example: Hydrogen cyanide, carbon monoxide and tar cause, or are associated with, cardiovascular disease and chronic obstructive lung disease, ammonia and formaldehyde cause eye, nose and throat irritations and other breathing problems (Hoffman *et al*., 2001). The main health risks in tobacco pertain to diseases of the cardiovascular system in particular myocardial infarction (heart attack), diseases of the respiratory tract by chronic obstructive pulmonary diseases (COPD), asthma, emphysema and cancer especially lung cancer, cancer of the larynx and tongue (Gillilang *et al*., 2006). These effects are considered to be due to the direct actions of tobacco-derived toxins and ciliotoxins causing connective tissue destruction, hyper secretion, pooling of mucus and blebbing of membranes of endothelial cells (Yates *et al*., 2001).

Among pregnant ladies who are exposed to cigarette, tobacco smoke reduces the delivery of oxygen to the foetus as a result of the presence of carbon monoxide, cyanide and aromatic hydrocarbons, nicotine and other substances in tobacco. Smoke causes reduction in placental blood flow creating further reduction in nutrients to the unborn baby. Second hand smoke exposure during pregnancy produce twice the risk of low birth weight babies (Foster *et al*., 2007).

1.4.6 Benefits of smoking

Cigarette smoking may also have some benefits. Recent studies suggest that smokers require less frequent repeated revascularization after percutaneous coronary intervention (PCI) (Cohen *et al*., 2001). Risks of ulcerative colitis have been frequently shown to be reduced by smoke on dose-dependent basis and this effect is eliminated if the individual stops smoking (Green *et al*., 2000). Despite the benefits of smoking, the dangers far outweigh the benefits and hence need for the study.

1.5 Factors that may affect cyanide levels in the body

Cyanide in air is in form of hydrogen cyanide. Cyanide is released to air through vehicles emissions and manufacturing industries (ATSDR, 1997). Chemical manufacturing and processing industries have been reported to release an estimated value of 1 million tonnes of HCN to air (ATSDR, 1997). Other non-industrial sources like agricultural pest control release 62 tonnes; incineration, 8.2-82 tonnes and tobacco smoke, 5.9-340 tonnes of HCN to air (Fiksel *et al.*, 1981). The HCN has been found following the combustion of a number of synthetic polymers (Sklarew and Hayes, 1984). The maximum yield of HCN per gram of polyurethane foam ranged from 0.37 to 0.93 mg under non-flaming conditions and from 0.5 to 1.02 mg under flaming combustion (Sklarew and Hayes, 1984). Off gas from the shale oil retorting process has been reported to emit HCN in range of 7- 44 mg/m^3 (Sklarew and Hayes, 1984). Forest fires smoke have been reported to emit HCN among other toxicants into air (Baud *et al*., 1991). Inhalation of fumes from all these sources may affect the levels HCN in the body of an individual.

Sources of cyanide in water bodies have been reported to be due to run-off from cyanide containing anti-caking salts used on roads and agricultural washout (ATSDR, 1997). Drinking of such water (untreated) may lead to cyanide exposure. In this study, only cyanide exposure in air and food will be considered.

1.6 Problem statement and justification

Some effects of cigarettes have already been studied. However, on smoking hydrogen cyanide is produced which has a lot of effects in the human body. Paralysis of legs, a disease called konzo, has been reported to arise due to too much consumption of cassava. However, accumulation of HCN due to cigarette smoke can also lead to the same disease or even cause death. People are not aware of these risks associated to cigarette smoking, it is important to monitor cyanide levels in the body though its metabolites such as thiocyanate.

Studies on thiocyanate content in various biological indicators (serum, plasma, saliva, blood and urine) have been carried out, but no such studies have been done in Kenya. Therefore, urine thiocyanate as a biomarker of cyanide exposure was selected for this study since it can be obtained in large volumes and measurements are simple and rapid to determine. Saliva has no constant flow and is available in small quantities. Blood is not easy to obtain since subjects fear for the test of HIV and AIDS. Logistics in tissues and blood are difficult.

1.7 Hypotheses

i. There is no significant difference between concentration of SCN in urine of smokers and non-smokers.

ii. Frequency and duration of cigarette smoking does not increase the level of cyanide in the body.

iii. Certain environmental factors as well as diet do not have an effect on the concentration of cyanide in the body.

1.8 Objectives

1.8.1 General objective

To use human urine as a biological indicator of cyanide exposure and determine other factors through a questionnaire that influence cyanide levels.

1.8.2 Specific objectives

i. To determine the concentration of thiocyanate in urine of smokers and non-smokers.

ii. To relate the frequency and duration of smoking on thiocyanate levels in the body by a questionnaire.

iii. To relate diet and other environmental factors (smoke and fumes) on the levels of thiocyanate in the body using a questionnaire.

1.9 Significance of the study and anticipated output

The information obtained in this study will be used to sensitize the public on levels and effects of cyanide in their bodies as a result of dangers associated with smoking. The government can use this information as a guideline to assist in coming up with new policies to minimize cyanide poisoning especially due to cigarette smoking and effects on passive smokers. This information will have a significant implication on the people's health status.

1.10 Scope and limitations

i. The study covered specified areas in some parts of Nairobi and Thika districts.
ii. A questionnaire was given to a selected group of smokers and non-smokers in the study area. However, not all respondents to questionnaires volunteered to give urine samples.
iii. There are many other types of tobacco smoking but only cigarette smokers were considered in this study.
iv. Factors that influence cyanide levels in the body may be many but only diet, number of cigarettes, period of smoking, occupation exposure, vehicle fumes, dispose of synthetic polymers and fuel for cookers were considered.
v. People who were on medication were not considered for this study.

CHAPTER TWO

LITERATURE REVIEW

2.1 Cyanide exposure

Cyanide is present in small quantities in the air, soil and water. At any given time our bodies contain fluctuating levels of cyanide in various forms. The general population is exposed to cyanide primarily by ingestion of food and water, and to a-lesser degree by inhalation. The cyanide content in unpolluted air averages between 0.180 and 0.187 mg/m^3 considering a ventilation rate of 20 m^3/day (ATSDR, 2004). The average intake of hydrogen cyanide from air is about 3.8 µg/day for the non-urban; non-smoking population (ATSDR, 2004). Cyanide levels in smoke from U.S commercial cigarettes range from 10 to 400 µg/cigarette for sidestream smoke (ATSDR, 2004). Mean cyanide concentrations have been reported for some food products for example lima beans were reported to have 0.10-0.17 mg/g (ATSDR, 2004).

2.2 Hydrogen cyanide (HCN)

Cigarette smoke as a source of cyanide exposure is of interest, it is a complex medium containing approximately 4000 different constituents separated into gaseous and particulate phases (Hoffman *et al*., 2001). Among the gaseous components is hydrogen cyanide which does the most damage to the heart and blood vessels. Hydrogen cyanide is highly toxic, for a man an oral dose of 0.5-3.5 mg/kg leads to immediate death as does inhaled HCN at concentration of about 270 mg/kg for a few minutes (Hoffman *et al*., 2001). The LD_{50} of inhaled HCN for rats is 200 mg/kg for 30 minutes exposure (Hoffman

et al., 2001). The HCN exerts its effect by inhibiting the cytochrome oxidase in the respiratory chain. Hydrogen cyanide does not cause cancer, but it increases the risk of other chemicals causing cancer by damaging cilia, it is cilliatoxic. These are tinny hairs lining the airways that help to clear toxins away. By killing cilia, hydrogen cyanide causes other dangerous chemicals to be stuck in the lungs and airways contributing to development of acute inflammatory and chronic obstructive lung diseases (Dalhamm, 1970).

Hydrogen cyanide is a colourless or pale blue liquid or gas with a faint bitter almond-like odour. Hydrogen cyanide is used primarily in the production of substances such as adiponitrile, methyl methacrylate, chelating agents, cyanuric chloride, methionine and its hydroxylated analogues, and sodium and potassium cyanide. The threshold limit value for HCN at the workplace was set at 10 mg/kg, the short term exposure level is 4.7 mg/kg and lethal dose of HCN is 1 mg/kg (WHO, 2004).

Hydrogen cyanide is generated when a fuel that contains cyanide decomposes. The production of HCN in a fire is dependent upon several factors that include the chemical composition of the material burning, the oxygen content in the room, the temperature of the combustion process, and the presence or absence of ventilation (Yates *et al*., 2001). Although most fuels contain carbon, and the atmosphere is 79% nitrogen, it is highly unlikely that cyanide could be created under normal fire conditions due to the conditions required to form the triple bond (Yates *et al*., 2001). Rather, the cyanide (CN) molecule must exist in the fuel prior to combustion, in order for hydrogen cyanide to be released during the fire. When a fuel that contains CN molecules begins to burn, hydrogen cyanide

is the most likely form of cyanide to be released in addition to carbon dioxide and nitrogen dioxide (Yates *et al*., 2001). Hydrogen cyanide then mixes with other toxic gases and by products of combustion like carbon monoxide and NO_2 to produce a highly toxic mixture of substances.

Some researchers have theorized that cyanide may work synergistically with other fire gases such as carbon monoxide to incapacitate and suffocate those exposed to fire smoke (Norris *et al*., 1986). Earlier review studies conducted in Paris and Dallas, concluded that high blood cyanide levels showed a direct relationship with the probability of death in smoke inhalation patients, suggesting that hydrogen cyanide may play a more significant role than carbon monoxide in smoke inhalation deaths (Silverman *et al*., 1988).

2.3 Thiocyanate

As assessed by smoking machine delivery, approximately 30 to 200 μg of HCN are delivered to the mouth of the smoker with each cigarette (Rickert and Robinson, 1981). Hydrogen cyanide is metabolized by the liver to thiocyanate. In addition to combustion gases, certain foods, particularly leafy vegetables and some nuts are sources of cyanide (Padmaja, 1995). Thiocyanate is also present in beer (Bottoms *et al*,. 1982). Thus, thiocyanate is present in non-smokers as well as smokers and may be particularly high in vegetarians. Thiocyanate is distributed in extra-cellular fluid and is eliminated slowly by the kidneys. Due to the slow excretion, the half-life of thiocyanate is long (about 14 days). The long half-life of thiocyanate means that there is little fluctuation in plasma

thiocyanate concentrations within a day or from day to day. Thus, the time of sampling is not critical.

On the other hand, a given level of thiocyanate reflects exposure to HCN in tobacco smoke over several weeks preceding the time of the sample. When a smoker stops smoking, it takes an estimated 3 to 6 weeks for thiocyanate levels to drop to that non-smoking individual (Rickert and Robinson, 1981). There is some overlap in levels between smokers and non-smokers. However, on the average, smokers do have levels two to four times higher than those of non-smokers (Cohen and Bartsch, 1980).

Serum or plasma levels of thiocyanate correlate significantly with the number of cigarettes per day (range of correlation 0.25-0.48) (Rickert and Robinson, 1981). Salivary concentrations of thiocyanate may also be used as a noninvasive biochemical marker of smoke exposure (Luepker *et al.*, 1981). Concentrations of thiocyanate in saliva vary as a function of salivary flow rate (Mucklow *et al.,* 1978). Thus, a close correlation between salivary and plasma thiocyanate concentrations depends on stimulating flow of saliva.

2.4 Absorption, distribution and excretion

Exposure to cyanides could be in various ways for example breathing air, drinking water, eating food or touching soils that contain the chemical. Absorption of cyanides occurs across both mucus membranes and intact skins (Ellenhorn and Barceloux, 1988). Hydrogen cyanide is rapidly absorbed by the gastrointestinal and respiratory tract; the liquid and possibly the concentrated vapor are absorbed directly through the intact skin

(Hartung, 1982; WHO, 1984). Hydrogen cyanide is more rapidly absorbed from the gastrointestinal tract than cyanide salts (WHO, 1984). Absorption of cyanide from smoke inhaled by cigarette smokers is inferred by higher plasma levels of thiocyanate (a metabolite) in smokers compared to non-smokers (WHO, 1984). Landahl and Herrmann (1950) reported that humans retained 57-77% of inhaled hydrogen cyanide in the lungs. Cyanides are moderately lipid-soluble and penetrate the epidermis readily. In addition, some cyanide compounds, such as potassium cyanide, have a corrosive effect on the skin that increases the rate of dermal absorption (NIOSH, 1976).

Toxic amounts of cyanides are absorbed with great rapidity through the bronchial mucosa and alveoli (ATSDR, 1997). Liquid cyanide compounds are easily absorbed through intact skin upon direct contact due to their lipid solubility and rapid epidermal penetration.

Following absorption, cyanide is rapidly distributed throughout the body by the blood. Cyanide enters erythrocytes and is found at low concentrations in normal human blood and other organs. The cyanide concentration is higher in red blood cells than in plasma by a factor of 2 or 3, reflecting cyanides tendency to bind to methaemoglobin (ATSDR, 1997). Higher plasma concentrations of thiocyanate were found in the umbilical cord blood of infants born to smokers compared with those born to non-smokers (US EPA, 1985). After non-lethal exposure, plasma cyanide levels tend to return to normal levels within 4-8 hours. The estimated plasma half-life is 20 minutes to 1 hour (Hartung, 1982). In cases of fatal oral poisoning, cyanide was detected in the brain, blood, kidney, stomach wall, liver, and urine (Ansell and Lewis, 1970). Gettler and Baine (1938) reported brain

and liver cyanide levels of 0.06-1.37 mg/100 g and 0.22-0.91 mg/100 g tissue, respectively, in four humans who ingested fatal doses of cyanide. Tissues cyanide levels in a human after inhalation of HCN were 0.75, 0.42, 0.41, 0.33, and 0.32 mg hydrogen cyanide/100 g in the lung, heart, blood, kidney, and brain, respectively (ECETOC, 2004). Elevated levels of cyanide were seen in erythrocytes and elevated levels of thiocyanate in the blood, liver, and kidneys of rats receiving food fumigated with hydrogen cyanide (Howard and Hanzal, 1955).

Results of a drinking water study with rats indicate that subchronic cyanide administration up to 160 mg/kg/day potassium cyanide for 13 weeks does not lead to saturation of cyanide detoxification pathways (Leuschner *et al*., 1991). According to Ellenhorn and Barceloux (1988), cyanides may accumulate in the body cells by binding to metalloproteins or enzymes such as catalase or cytochrome C oxidase. Research shows that small but significant levels of cyanide are present in normal, health human organs at concentrations of < 0.5 mg/kg owing to the breakdown of cyanogenic foods and tobacco smoke by bacterial action and vitamin B_{12} (Feldstein and Klendshoj, 1954).

The principal pathway of cyanide metabolism is conversion to thiocyanate catalyzed by either rhodanese (thiosulfate sulfurtransferase) or by 3-mercaptopyruvate sulfurtransferase. Both enzymes are widely distributed in the body. Conversion of cyanide to the less toxic thiocyanate by rhodanese is enhanced when cyanide poisoning is treated with the intravenous administration of a sulfur donor such as sodium thiosulfate (Westley, 1980). The toxicity of thiocyanate is significantly less than that of cyanide, but chronically elevated levels of blood thiocyanate can inhibit the uptake of iodine by the

thyroid gland, thereby reducing the formation of thyroxine (Hartung, 1982). Other metabolic pathways include the conversion to 2-aminothiazoline-4-carboxylic acid; incorporation into a 1-carbon (formate) metabolic pool; combination with hydroxycobalamine to form cyanocobalamine (B_{12}); and combination with cystine to form 2-aminothiazoline-4-carboxylic acid (ATSDR, 1989).

In humans and animals, the major route of cyanide elimination from the body is via urinary excretion of thiocyanate. Small amounts of thiocyanate are also eliminated via lung and feaces (US EPA, 1985). Some free hydrogen cyanide is excreted unchanged in breath, saliva, sweat, and urine (Hartung, 1982). An increased urinary excretion of thiocyanate was observed in people exposed to 4-6 ppm cyanide vapor and cyanide salts over a period of several years (NIOSH, 1976).

According to Agents for Toxic Substances and Diseases Registry (ATSDR, 1997), an average urinary concentration of thiocyanate normally ranges between 0.85 and 14 mg/L over 24-hour period. Some cyanides are metabolized directly in the body, carbon dioxide and hydrogen cyanide are eliminated. Figure 2.0 shows the processes involved in metabolism of cyanides.

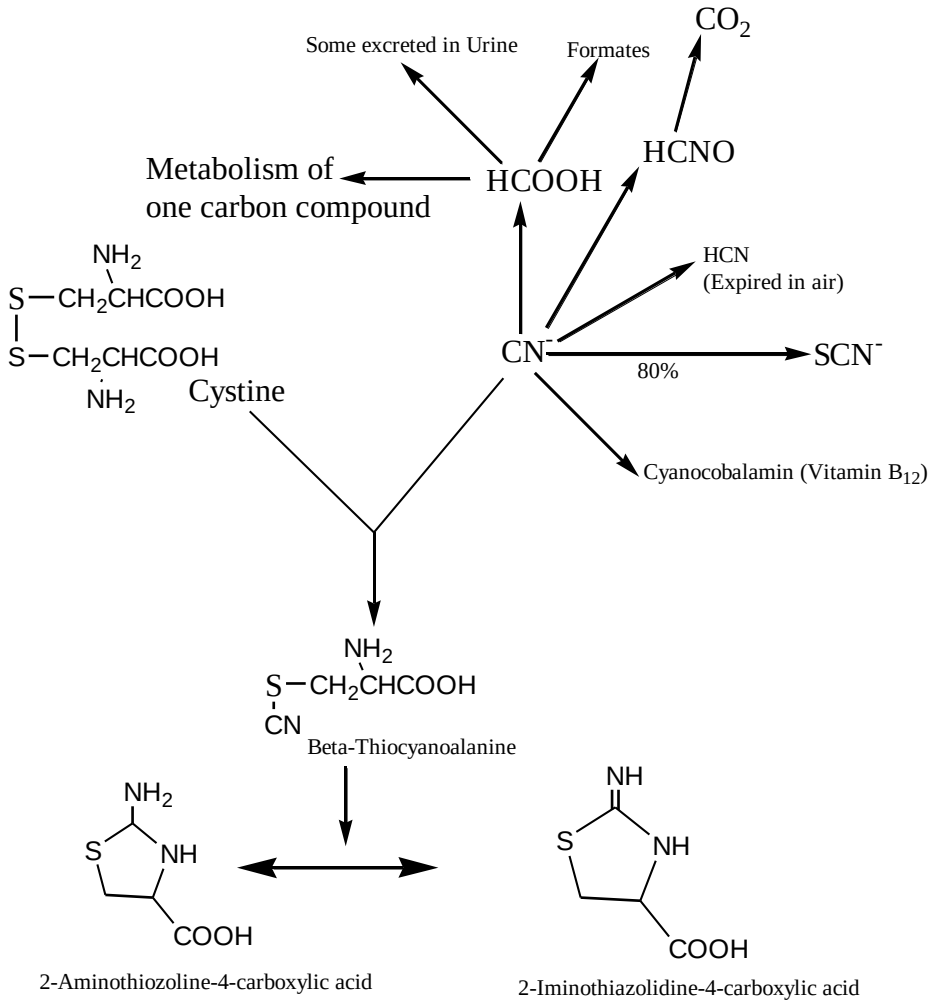


Figure 2.0 Basic products involved in the metabolism of cyanide

Source: ATSDR, 1997

2.5 Toxic effects

Cyanide is highly toxic to living cells and causes inhibition of growth by at least three major mechanisms: tight chelating to di- and trivalent metals in metallo-enzymes such as cytochrome oxidase hence inhibition of cellular respiration; reaction with keto compounds to form cyanohydrin derivatives of enzymes substrates; and reaction with Schiff-base intermediates during enzymic reaction to form stable nitrile derivatives

(Solomonson, 1981). The toxic effects of cyanide ion in humans and animals are believed to result from inactivation of cytochrome C oxidase and inhibition of cellular respiration and consequent histotoxic anoxia.

The primary target for cyanide toxicity in humans and animals are cardiovascular, respiratory, central nervous system and endocrine system, since long term toxicity due to exposure to thiocyanate prevents uptake of iodine in the thyroid and acts as goitrogenic agent. Reports from US Environmental Protection Agency US EPA (1987), show that the lethal oral dose of cyanide compounds to humans generally ranges from 50 to 200 mg cyanide (0.72 to 2.9 mg/kg body weight).

In humans, slight effects occur due to exposure of cyanide 20 to 40 mg/m^3, 50 to 60 mg/m^3 can be tolerated without immediate or late effects for 20 minutes to one hour, 120 to 150 mg/m^3 may lead to death after 0.5 to one hour while 200 mg/m^3 is likely to be fatal after 10 minutes (Gettler and Baine, 1938). According to WHO, (1984), low exposure to cyanides is not fatal to humans with efficient detoxification systems; however, exposure to low levels of cyanides over a long period results in increased blood cyanide levels. This may result in weakness of fingers and toes, difficulty in walking, dimness of vision, deafness and decreased thyroid gland function. Skin contact with cyanide can produce irritation and sores (Setting, 1985).

Symptoms of moderate poisoning include vomiting and nausea, convulsions, deep breathing, shortness of breath and anxiety and restlessness, dizziness, weakness and

headache. More serious cases result in convulsions, loss of consciousness and death after apnea and cardiac arrest hypoxemia, low blood pressure, lung injury and slow heart rate.

2.6 Mechanism of toxicity

Cyanide has a high affinity for certain sulfur compounds like sulfanes and certain metallic complexes, particularly those containing cobalt and the trivalent form of iron (Fe^{3+}) like cobalt EDTA and methemoglobin respectively. The cyanide ion can rapidly combine with iron in cytochrome oxidase complex in mitochondria to inhibit this enzyme, thus preventing intracellular oxygen utilization. The cell then utilizes anaerobic metabolism, creating excess lactic acid and a metabolic acidosis. Cyanide also has a high affinity for the ferric iron of methemoglobin, and one therapeutic stratagem induces the formation of methemoglobin to which cyanide preferentially binds (Ellenhorn and Barceloux, 1988). The cyanide ion therefore, kills all aerobic organisms by shutting down the respiration in cells, impaires both oxidative metabolism and the associated process of oxidative phosphorylation (Holland, 1983).

2.7 Treatment of cyanide poisoning

Therapies for cyanide poisoning convert part of the hemoglobin of the blood from ferrous (+2 charge) hemoglobin to ferric (+3 charge), this creates a pull of binding potentials that can divert cyanide from the cytochrome it poisons. The antidotes normally used are briefly discussed in the following subsections.

2.7.1 Sulfur donors

Cyanides from natural sources inside the human body can usually be handled effectively through sulfur detoxification. This is mediated by mitochondrial rhodanese and B-mercaptopyruvate sulfur transferase inside the body and thiocyanate is the end product. The latter is much less toxic and easily excreted in urine. With excessive absorption of cyanides into the body, the natural substrates become depleted rapidly. A therapeutic approach is to introduce sulfur donors into the body to fuel the detoxification process (Sylvester *et al*., 1983).

2.7.2 Methemoglobin inducers

The high affinity of cyanide for iron in the ferric state in methemoglobin (metHb) results in the formation of cyanomethemoglobin. If the methemoglobin concentration in blood is increased, it reduces the binding of cyanide to the cytochrome oxidase system. Cyanide is then slowly released from cyanmethemoglobin and diffuses out of red blood cells to be detoxified by hepatic rhodanese and other sulfur transferase systems. This process is facilitated by exogenous sources of sulfur. Excessive metHb production of >40% poses significant risk as inadequate normal hemoglobin would be left for the transport of oxygen in the blood. Conventional methemoglobin inducers used for treating cyanide poisoning include amyl nitrite and sodium nitrite. The former can be administered through inhalation by anyone with minimal training and is often preferred in the first-aid management of cyanide poisoning. Sodium nitrite must be administered intravenously. The administration of methemoglobin inducers is usually followed by sodium thiosulfate (Pettersen and Cohen, 1985).

2.7.3 Cobalt compounds

Cobalt compounds are given to treat cyanide poisoning because cyanide combines directly with cobalt compounds. The most common form is dicobalt edetate (cobalt EDTA or Kelocyanor), which is more effective than the combination of sodium nitrite and sodium thiosulfate. The diagnosis of cyanide poisoning must be established before using this agent due to the inherent toxicity of cobalt in the absence of cyanide in the body. The toxicity is reduced by co-administration of glucose (50 ml of 50% by intravenous infusion). This agent should not be used in doubtful or mild cases (Rose *et al*., 1965).

2.7.4 Hydroxocobalamine

An agent used in Europe is hydroxocobalamine (Vitamin B12a), which combines directly with cyanide to form cyanocobalamin (Vitamin B12). It has low toxicity and rapid action, and is theoretically an ideal antidote. On the other hand, it has a high molecular weight and combines with cyanide on a 1:1 molar basis, necessitating the administration of a relatively large dose. Furthermore, most medical preparations are in the form of 1-2 mg (1.2 ml) ampoules, so large volumes are required for a sufficient dose and intravenous infusion of the solution is necessary (Way *et al*., 1984).

The choice of an antidote depends on the extent of poisoning, for mild poisoning**,** decontamination followed by rest and oxygen may be adequate. Any deterioration is an indication for amylnitrite, and transfer to hospital should be arranged. Symptoms might be delayed with some compounds, such as acetonitrile, which only release cyanide upon metabolism. For moderate poisoning, cases whose symptoms include brief periods of unconsciousness, convulsions or cyanosis, intravenous administration of antidotes is

indicated and they are best managed in a hospital, ideally in the intensive care unit. Sodium thiosulfate may be first choice if the diagnosis is uncertain. For severe poisoning, in- patients with deep coma, dilated non-reactive pupils and worsening cardio-respiratory function, an additional intravenous antidote is required. The choice depends on many factors including the experience of the treating physician and the availability of the preparations. Since metHb inducers and cobalt EDTA both have potentially serious side effects, hydroxocobalamin is the best choice from a risk-benefit point of view. Cyanide poisoning in the body makes it necessary for monitoring cyanide exposure by use of its metabolites in high risk groups such as smokers.

2.8 Human body fluids as biological indicators for cyanide poisoning

The thiocyanate ion is usually present in low concentration in human urine, serum and saliva as a result of food consumed containing cyanogenic glycosides. Blood, serum, plasma and urine have been employed as indicators of cyanide exposure in humans (Lauwerys and Hoet, 2001). However, at low levels of occupational exposure, the relationship between exposure and urinary thiocyanate concentrations shows a wide inter- and intra-individual variation due to a variety of factors (such as diet, period of smoking, exposure to vehicle fumes and cigarette smoke).

A Study on thiocyanate (SCN) in urine showed that smokers had an average concentration of 0.155 mg/L in urine while non–smokers had 0.075 mg/L of thiocyanate in urine (Jarvis *et al*., 1984b). Due to the relatively high salivary SCN concentrations (about 20 times higher than in plasma) and the noninvasiveness of saliva collection, SCN is frequently determined in saliva. However, a number of factors may influence the

determination of SCN in saliva, thus increasing the variability of this biomarker compared to SCN in plasma or urine (Prue *et al*., 1981b). In another study, SCN in saliva was found to be more stable than SCN in plasma or urine (Prignot, 1987). Table 2.1 shows mean thiocyanate levels of smokers and non-smokers in body fluids (serum/plasma, urine and saliva) as reported by various researchers.

Table 2.1: Mean SCN concentrations in body fluids of smokers and non-smokers

Study	**Non-smokers (*N*)**	**Smokers (*N*)**
SCN in serum or plasma (µmol/l)		
Bliss and O'Connell (1984) (Review: Weighted average of 19 studies)	59.70 ±41.10 (6815)	156.46 ±58.62 (10377)
Clark *et al*. (1981)	0.06	0.17
Cohen and Bartsch (1980)	73.5 ± 48.3 (191)	180.2 ±55.7 (426)
Jarvis *et al*. (1984b)	50.8 (100)	122.9 (94)
Fortmann *et al*. (1984)	53.1 ± 25.6 (970)	163.7 ± 49.6 (543)
Nikitin *et al*. (1990)	Males: 42.8 ± 22.4 (706) Females: 41.0 ± 720.9	Males: 116.6 ± 43.3 (1025 Females: 122.9 ± 49.0 (55)
Ockene *et al*. (1987)	54.4 ± 30.1 (1356)	Cigarette smokers: 173.8 ± 55.4 (5090) Primary cigar/pipe smokers: 90.27 ± 51.4 (414) Secondary cigar/pipe smokers: 111.67 ± 66.3 (497)
Ruth and Neaton (1991)	53.0 ± 27.3 (3274)	172.8 ± 52.2 (4553)
Assaf *et al*. (2002)	Men: 60.6 (493) Women: 43.0 (850)	Men: 137.9 (496) Women: 141.3 (507)
Korpilahde *et al*. (2004)	Never smoked:5.3±6.2 (3776) Ex-smokers: 7.4±9.6 (1417)	Only pipe or cigar: 29.3 ± 20.2 (85) Cigarettes and pipe/cigars: 31.1 ± 19.9 (63) 1–9 cig/d: 23.0 ± 16.1 (351) 10–19 cig/d: 31.4 ± 17.6 (577)

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Study	Non-smokers	Smokers
SCN in saliva (µmol/l)		
Bliss and O'Connell (1984) (Review: Weighted average of 11 studies)	1219 ± 757 (242)	2724 ± 1112 (287)
Haley *et al*. (1983)	1293 ± 652 (18)	3339 ± 1117 (12)
Jarvis *et al*. (1984b)	1300 (100)	2450 (94)
Degiampietro *et al.* (1987) Induced mixed saliva, parotid saliva: ca. 40% higher concentrations	Median: 1670 (207)	Median: 2920 (117)
SCN in urine (µmol/l)		
Densen *et al*. (1967)	285 ± 30 (6)	560 ± 35 (13)
Jarvis *et al*. (1984b)	74.8 (100)	154.9 (94)
Lundquist *et al*. 1995	0.043 ± 0.022 mg/ L	0.057 ± 0.00 mg/L
Muranaka *et al.* (1988)	(µmol/g creatinine) 77.0 ± 20.6 (42)	(µmol/g creatinine) 132.9 ± 52.0 (67)
Bradbury *et al*. 1999	11±0.12 mg/ L	

Source: Scherer, 2006

2.9 Methods of analysis

Well established standard analytical methods are available for detecting, measuring and monitoring cyanides, its metabolites and other biomarkers of exposure and effects of cyanides. Cyanide in environmental media are usually collected in sodium or potassium hydroxide solution and measured by colorimetric methods (Agrawal *et al*., 1991), colorimetry or ion specific electrode or by headspace gas chromatography with nitrogen specific detector or electron capture detector (Seto *et al*., 1993). Photometry has also been used for cyanide detection in earlier studies (Butts *et al*., 1974; Bhide and Jayant, 1987; Degiampietro *et al*., 1987). Cyanide in aqueous matrices is usually measured by

colorimetric, micrometric or electrochemical methods after pretreatment to produce hydrogen cyanide and absorption in sodium hydroxide solutions (US EPA, 1983). Total cyanide irrespective of the source is measured by semi-automatic colorimetric as well as selective electrode ultraviolet/distillation/spectrophotometer and ion chromatography.

Free cyanide can also be determined by specific ion electron method. A chromatographic technique with fluorescence detection is used to detect trace amounts of cyanides in blood cells (Chinaka *et al*., 1998). Four principle methods are used for the determination of SCN in body fluids: (i) Ko'nig reaction: dye formation by reaction of SCN with an aromatic amine, bromocyanide (BrCN) and pyridine (Bhide and Jayant, 1987); (ii) dye formation of SCN with Fe (III) nitrate (Butts *et al*., 1974; Degiampietro *et al*., 1987); (iii) colorimetry (Walters and Sawhney, 1987; Michigami *et al*., 1992); (iv) gas chromatography with mass spectrometry (GC-MS) after derivatization (Torano and van Kan, 2003).

In colorimetry, the intensity of absorption depends on the concentration of the absorbing species C (for this study it is the thiocyanate), and the path length traversed by the radiation *l.* The molar extinction coefficient ϵ, is a measure of the efficiency with which the molecules absorb. This is defined by Beer- Lamber's law as,

$$\epsilon = 1/Cl \quad \log_{10} I_0 / I \quad \text{or} \quad A = \epsilon\, cl \ldots\ldots\ldots\ldots \text{Eq } 2.0$$

A is log of ratio of the intensity of the incident (I_0) and transmitted (I) radiation is called the absorbance or optical density

2.9.1 Picrate paper method

Bradbury *et al*. (1999), developed a kit to determine thiocyanate content in urine among cassava consumers to help monitor cyanogen overload hence predict the possible onset of a condition called konzo.

Picrate paper method is based on oxidation of SCN with acidified permanganate to sulphate and cyanide ion as shown in equation 2.1 (Egan *et al*., 1998).

$$6\,MnO_4^-{}_{(aq)} + 5\,SCN^-{}_{(aq)} + 13H^+{}_{(aq)} \longrightarrow 6\,Mn^{2+}{}_{(aq)} + 5\,HCN_{(g)} + 5\,SO_4^{2-}{}_{(aq)} + 4H_2O_{(l)} \quad \text{....Eq 2.1}$$

The hydrogen cyanide gas formed then reacts with picric acid to form a charge-transfer complex that is brown in colour. These complexes are characterized by one component that is electron-rich (π-donor) such as CN^- and another component that is strongly electron attracting (π-acceptor) such as picric acid. They are also known as donor-acceptor complexes. The complexes are characterized as a hybrid of two resonance structures as shown in Figure 2.1.

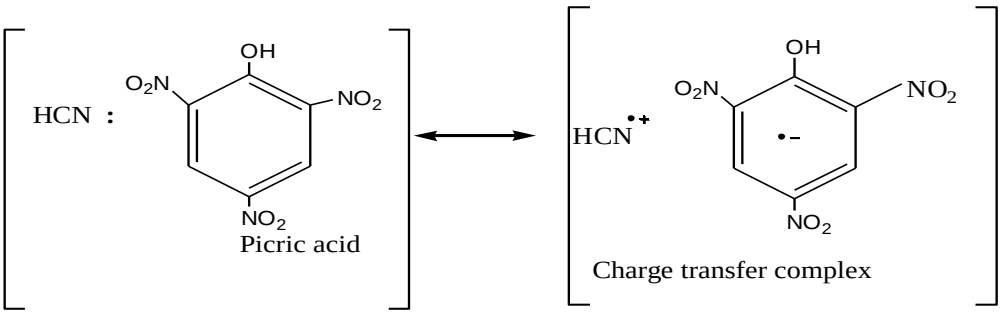

Figure 2.1: Resonance structure of charge transfer complex between picric acid and HCN

Source: Andrew *et al*., 1985

Charge-transfer complexes are often intensely coloured. The colour is associated with an electronic transition in which a substantial fraction of an electron is transferred with absorption of energy and the intensity of absorption depends on the beers lambert's law which is the principle behind spectrophotometric technique. Picrate paper method by Bradbury *et al*. (1999) was used more in this study. Paper picrate method is simple, requires cheap and readily available chemicals and equipment as well as availability of a kit sent by Bradbury himself, which can be used in the field study with limited equipment.

2.9.2 Lundquist method

This is a general procedure developed and used by Lundquist *et al.* (1995), it is based on the high affinity of SCN to a weakly basic anion –exchange resin. Thiocyanate is thus separated from interfering compounds then eluted using KNO_3. SCN^- is then determined with a new modification of the ko'ning reaction , where sodium hypochlorite is used for halogenation and barbituric acid is used as the coupling agent for complex formation (pyridine dye stuff) (Lundquist *et al*., 1995). The SCN is first converted to cyanogen halide with sodium hypochlorite. On addition of a mixture of pyridine –barbituric acid, a coloured charge transfer complex is formed hence determined spectrophotometrically at 580 nm.

CHAPTER THREE

METHODOLOGY AND EXPERIMENTAL

3.1 Research design

The research design in this study was in three parts. The first part entailed sensitization of the people in the study area on the purpose of study. The second part involved a descriptive survey by use of a questionnaire (see appendix III). The last part entailed collection of questionnaires, samples from the subjects and experimental laboratory analysis by use of colorimetric method. Preparation and analysis of urine samples was carried out using standard methods as reported by Bradbury *et al.* (1999) and Lundquist *et al.* (1995).

3.2 Study area

Nairobi, the capital city of Kenya, covers an area of 700 km^2 with a population of over 2.1 million (CBS, 2000). Thika district is situated in central province of the republic of Kenya with a population of 645,713 and covers an area of 1,960.2 km^2 (NCAPD, 2005). Figure 3.0 shows a map of study areas in Nairobi and Thika. The study sites included: Kahawa-wendani, Kahawa-sukari, Githurai, Kenyatta University, Ruiru and Kiwanja as shown in figure 3.0. The study sites were chosen based on accessibility, convenience hence it was easy to follow up the subjects for subsequent sampling and questionnaire collection. The subjects recruited were either male or female, who were residents of the area of study, aged between 20 and 70 years.

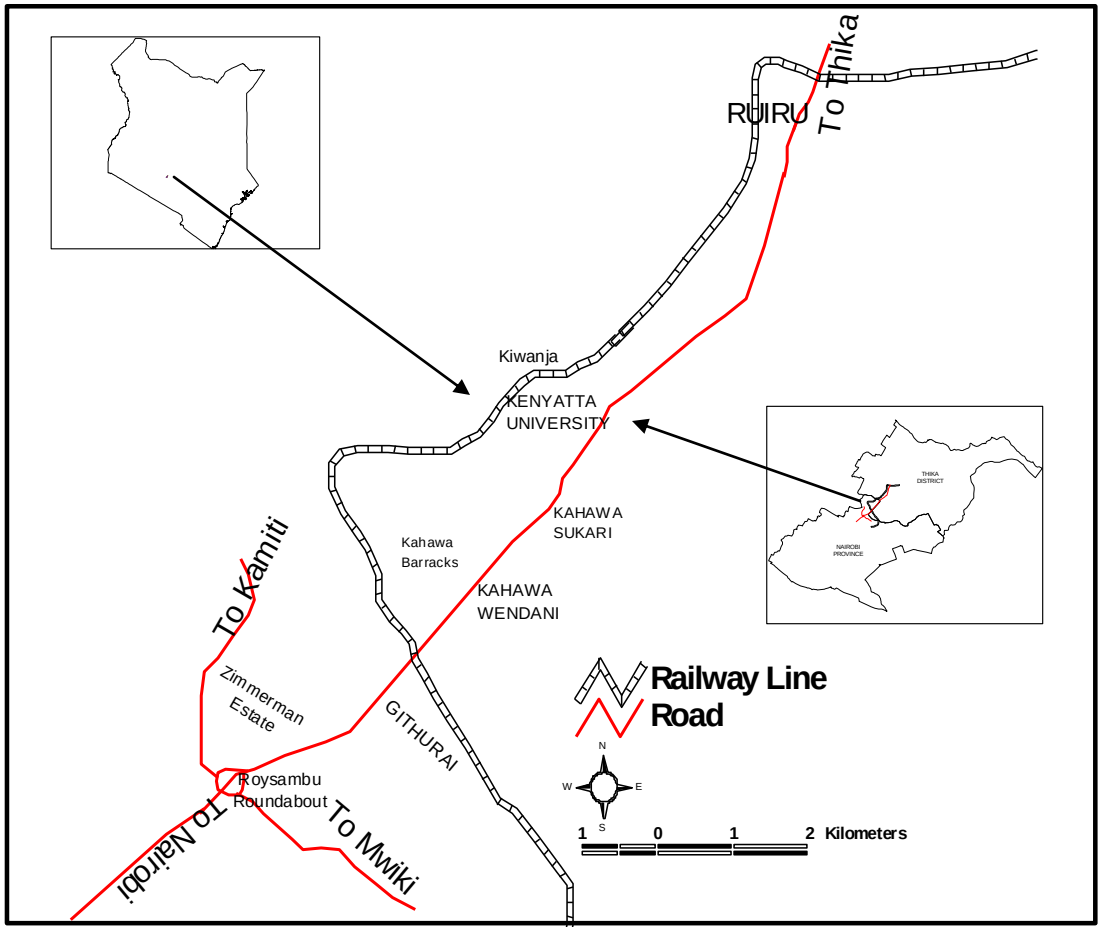

Figure 3.0: Map showing study areas in Nairobi and Thika

(Kahawa Wendani, Kahawa Sukari, Githurai, kiwanja, Kenyatta University and Ruiru*)*

3.3 Ethical consideration

A permit to carry out the research was obtained from the Ministry of Education, Science and Technology; see appendix V. Having been granted an approval, the subjects were recruited with the help of persons who were familiar with the areas of study. The subjects were informed about the research, expected outcome and confidentiality of results. Those who accepted were given the questionnaires and sample bottles.

3.4 Sample size and sampling procedure

Purposive sampling strategy was used to select sampling sites and subjects mainly around Kenyatta University. The sample size was calculated using the formula reported by Daniel (1999):

$$n = \frac{Z^2 \; P(1 - P)}{d^2} \quad \text{..... Eq 3.0}$$

Where n = sample size

Z = Z Statistic for a level of confidence (for the level of confidence of 95%, which is convectional, Z value is 1.96)

P = Expected prevalence or proportion (in proportion of 75%, $P = 0.75$)

d = precision (in proportion of 12.5%, $d = 0.125$)

$$n = \frac{1.96^2 \left[0.75 \; (1 - \; 0.75) \right]}{0.125^2} \quad \text{..... Eq 3.1}$$

$$n = 46.09$$

Therefore from each of the six area of study; 46 subjects were randomly recruited, 23 of which were non-smokers. A total of 276 samples were collected per sampling session to give a representative sample. Each of the recruits filled a questionnaire once and gave at least 10 ml of urine per sampling session. There were only two sampling sessions. Each subject was issued with a pair of clinical gloves (to minimize infections), a roll of tissue paper, and a clean, corked and labeled sample bottle for urine collection. The filled questionnaires and sample bottles were collected from each volunteer smoker and non-smoker at designated places. A total of 301 samples were excluded from this study in the entire period since some of them failed to fill questionnaires, could not be traced for

preceding sample collection and their urine spilled out or they failed to give samples. Only two hundred and fifty one (251) samples were obtained from the same individuals for the whole sampling session. The urine samples were then refrigerated at -4^0C awaiting laboratory analysis. The SCN content in urine is stable for 6 months.

3.5 The questionnaire

Volunteer smokers (n = 128) and non-smokers (n = 123) in the study areas filled in the questionnaire. The questionnaire elicited information on the period of smoking, reasons for smoking and number of cigarettes one consumed, some dietary habits of the subjects as well as environmental risk exposure factors. The dietary habits considered were consuming foods with high cyanogenic content like cassava and sorghum. Environmental exposure considered include working in industries where cyanide is used like metal processing industry, plastics, pesticides and rodenticides processing industries. Exposures to fire smoke from burning synthetic polymers, firewood/ charcoal/ paraffin cookers and exposure to second hand smoke were also considered for potential cyanide exposure. The subjects were guided before filling the questionnaire and those who did not understand English were interviewed in their local dialects through interpreters.

3.6 Laboratory procedures

3.6.1 Equipment, chemicals and reagents

The major equipment used in this study was UV-visible spectrometer, Cecil CE 2041 2000 series from England and the electronic analytical balance, AAA model from Britain. The reagents were of high quality, analytical grade. These included; sulphuric acid, potassium permanganate, nitric acid, hydrochloric acid, picric acid, sodium carbonate,

barbituric acid, pyridine, acetic acid, potassium nitrate, Picrate paper for thiocyanate determination courtesy of Prof Bradbury (Australian National University), distilled-deionised water resin (amberlite resin IR-45 (OH) of 100-200 mesh) and potassium thiocyanate. They were supplied by Loba chemie from India.Water distillation was done by use of distillation machine from Great Britain of the model WSB/4 and deionization was done using Elegastat micromeg 1190 model from England.

3.6.2 Cleaning of glass ware and sample containers

All sampling containers and glassware were cleaned in a non-ionic liquid soap (Laser clean from Laser Chemicals International) and soaked in 5% nitric acid for 24 hours to remove adsorbed metal ions which may react with cyanides to form metal cyanide salts. They were then rinsed with distilled-deionised water before drying in the oven at 105^0C. The dry apparatus were safely stored in clean drawers awaiting usage.

3.6.3 Preparation of reagents

3.6.3.1 Barbituric-pyridine reagent

To a 250 ml beaker, 6.0 g of barbituric acid was dissolved in a mixture of 30 ml pyridine and 64.0 ml of distilled-deionised water in which 6.0 ml concentrated hydrochloric acid was added. The solution was put into a clean plastic container and stored in a refrigerator (-4^0C). This reagent is stable for one week in a refrigerator (Lundquist *et al*., 1995). Therefore, this regent was prepared weekly.

3.6.3.2 Sodium hypochlorite (50 mmol/L)

Using a pipette, 5 ml of 0.5 M NaOCl were diluted in 0.1 M NaOH solution to a final volume of 50 ml using a 50 ml volumetric flask. This reagent is stable for one month in a refrigerator (-4^{0}C (Lundquist *et al*., 1995). Therefore, this regent was prepared monthly.

3.6.3.3 Anion exchange resin (amberlite resin IR-45 (OH))

The resin, amberlite resin IR-45 (OH) of 100-200 mesh, was washed with water and suspended in 1 M HCl. It was then filtered using suction pump and washed with water until washings attained a pH equal to 4.5 or greater. The resin was then oven dried at 100^{0}C for 12 hrs. It was then suspended in water for 15 min thereafter resuspended in an equal volume of 1 M sodium hydroxide after decanting. After 15 min, the resin was washed with water until the washings attained a neutral pH of 7.000. The pretreatment of the resin was done to lower blank values.

3.6.3.4 Picrate papers

Using a watch glass, 1.4 g of moist picric acid (used as coupling agent) was weighed and added to 100 ml of sodium carbonate solution made by dissolving 2.5 g of sodium carbonate in 100 ml of distilled water. Using Whatman 3 MM filter paper supplied in the Bradbury kit, 10 cm by 10 cm square papers were cut and placed in the picrate solution for 20 seconds before being air dried. Unevenly coloured sections were cut off and the remaining papers cut into 3 cm by 10 cm rectangular pieces. Plastic strips (5 cm by 1 cm) were glued on picrate papers using vinyl acetate glue. The picrate papers were stored in a deep freezer to ensure stability.

3.6.3.5 0.1M standard potassium thiocyanate stock solution

Potassium thiocyanate stock standard solution was prepared by accurately weighing 1.6732 g of KSCN into a 1000 ml volumetric flask and diluting with distilled water up to the mark. Working solutions were prepared weekly by serial dilutions and checked for consistency of absorption before taking the readings. This was used to draw calibration curves (see appendix II, figure A1 and figure A2)

3.6.4 Determination of thiocyanate using picrate paper method

Using a 1 ml plastic pipettes 1.0 ml urine was placed in a flat bottomed plastic bottle followed by 3 drops of 1 M H_2SO_4 using a second 1 ml plastic pipette then mixed. Three drops of 0.1 M $KMnO_4$ were added to the mixture and immediately a yellow picrate paper attached to a plastic strip was put on the bottle carefully ensuring that the picrate paper doesn't touch the liquid in bottle. The bottle was then closed immediately with screw capped lid and the solution gently mixed and allowed to stand for 24 hrs at room tempereture. This procedure was repeated for blank and 10 mg/L standard solutions. The bottles were then opened and the colour on the picrate papers was matched with that of the colour chart provided in Bradbury picrate paper kit (see appendix IV). This gave an estimate of thiocyanate content in parts per million (mg/L) see appendix I, table A1.

To obtain the actual concentration of SCN in the samples, picrate solutions were made by placing picrate papers in testubes containing 5 ml of deionised water. After 30 min, the absorbance of picrate solutions was obtained at 510 nm using UV-visible spectrometer (Bradbury *et al.*, 1999) (see appendix I, table A2). A linear calibration curve ($r^2 = 0.9963$) for thiocyanate was prepared using AR-grade KSCN (see appendix II, figure A2).

Working solutions over the range of concentrations from 0 to 100 mg/ L were made from the stock solution by serial dilution and treated like the samples. The absorbance obtained at each thiocyanate value were averaged and used to draw a calibration curve from which SCN content of urine samples were determined. Adequate quality control was ensured by analysing standards and blank samples using above described procedure after every ten samples analysed and carrying out two replicate analyses.

3.6.5 Thiocyanate determination by Lundquist method

Fifteen samples were randomly picked for analysis using this method. A 0.5 ml aliquot of urine were diluted with 5.0 ml of 0.1 M NaOH and applied to a glass column (2.5 cm by 0.7 cm i.d) of activated amberlite resin IR-45 (OH), 100-200 mesh. The column was then washed three times with 5 ml of distilled water. This washing was to eliminate antibiotics in urine that may interfere with measurements of thiocyanate. The adsorbed thiocyanate was eluted with 8.0 ml of 1 M KNO_3. To 4 ml of the eluate, 0.2 ml of 0.5 M acetic acid was added and mixed. 0.1 ml of 50 mmol /L sodium hypochlorite was then added and mixed. Within one minute, 0.5 ml of the barbituric acid-pyridine reagent (used as coupling agent) was put into the mix and mixed thoroughly. 10 minutes later, absorbance was determined at 580 nm verses the blank using UV-visible spectrophotometer (see appendix I, table A2).

The thiocyanate concentrations in the sample were read from a calibration curve prepared from SCN standards of known concentrations (see appendix II, figure AI). Because a 0.5 ml urine sample was used, the amount of thiocyanate in 1 ml of urine is equal to twice the

value obtained. The resin was cleaned and activated using 30 ml of 6 M NaOH solution and the eluate was tested for nitrates using the brown ring test. This was repeated until no more brown ring was observed.

3.6.6 Comparison of chromatographic paper and Whatman 3 MM paper for SCN determination

There is need to compare whether using chromatographic paper and Whatman would give same results, as chromatographic paper was readily available and cheaper. A grade one chromatographic paper was cut into 10 cm by 10 cm square papers and treated in picric acid under the same conditions as Whatman 3 MM paper. The treated papers were cut into 3 cm by 1 cm rectangular papers and glued to plastic strips (1 cm by 5 cm). The analysis was repeated using chromatographic paper and results compared.

3.7 Data analysis

Test of significance of thiocyanate levels was determined by ANOVA and t-test. The student t-test was used to compare the concentration of thiocyanate in urine among smokers and non-smokers. Correlation coefficient was used to compare the two methods of analysis. All the calculations were done using statistical SPSS program (11.5). The significance level was set at $P = 0.05$.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Introduction

Samples of one hundred and twenty eight (n =128) smokers and one hundred and twenty three (n = 123) non-smokers, randomly recruited from six sampling sites were analyzed in triplicate for thiocyanate concentration. The mean results of the individual analysis are given in table A1, appendix I. However, the UV results using the picrate paper method were adopted as they were more accurate than the colour chart results. The statistical results from experimental analysis of thiocyanate levels in urine samples are presented in Tables 4.0 – 4.10 and Figure 4.1 – 4.3.

4.2 Comparison of SCN levels in subjects regardless of the study areas

Mean SCN levels (mg/L±SE) of smokers and non-smokers were compared irrespective of the sampling site of the sample. The results of comparison are shown in Table 4.0.

Table 4.0: SCN levels in urine samples of smokers and non-smokers regardless of sampling sites along with t-test at p = 0.05

Respondent	N	Mean±SE (mg/L ± SE)	Range (mg/L)	t calc	P value	df	Mean diff ± SE
Smokers	128	3.89±0.17	0.88-11.95	8.99	<0.05	230	1.90±0.21
Non-smokers	123	1.99±0.12	0.03– 6.75				

From the results in Table 4.0, a higher mean level of 3.89 ± 0.17 mg/L thiocyanate with a range of 0.88 –11.95 mg/L was obtained in urine samples of smokers compared with a mean of 1.99 ± 0.12 mg/L for non-smokers with a range of 0.03 – 6.75 mg/L. Comparing the levels of SCN obtained for smokers and non-smokers using t-test, a statistically significant difference (P <0.05) in the levels was observed. This Earlier research studies

reported that thiocyanate in smoker's urine were about 2- 3 times higher than in non-smokers (Cohen and Bartsch, 1980). The differences in the levels could be attributed to cigarette smoke and numerous other sources of cyanide like diet and vehicle exhaust (Baumerster *et al*., 1975; Padmaja, 1995). Non-smokers also have SCN due to the foods they consume or second hand tobacco smoke (Schick and Glantz, 2006). The results agree with those of Chandra *et al*. (1980) who revealed that smokers had an average concentration of 3.2 mg/L in urine while non-smokers had 2.16 mg/L of SCN in urine.

4.2.1 Comparison of mean SCN content in urine of smokers and non-smokers from study site

The mean SCN concentrations (mg/L±SD) in urine of the smokers and non-smokers were compared per sampling site at 95% confidence level using one-way ANOVA. The results are given in Table 4.1

Table 4.1: Mean SCN levels in urine of smokers and non-smokers from study areas

Site	**Smokers**				**Non-smokers**			
	N	Mean±SD (mg/L)	Range	Pvalue	N	Mean±SD (mg/L)	Range	P value
Ruiru	21	5.34±3.13	1.05-11.95	0.50	21	1.87±1.31	0.34-4.03	0.99
Githurai	23	4.19±1.51	1.25-6.47	0.50	20	2.17±1.58	0.33-6.75	0.99
Kahawa sukari	20	3.12±1.24	1.29-5.37	0.01*	21	1.50±1.24	0.03-4.60	0.98
Kahawa wendani	21	3.91±1.84	1.22-7.49	0.27	19	2.11±0.92	0.36-4.05	1.00
Kenyatta University	22	3.55±1.30	0.88-6.07	0.07	21	2.17±1.74	0.07-6.70	0.99
Kiwanja	21	3.21±1.16	1.37-5.36	0.02*	21	2.14±1.38	0.08-4.79	1.00

P values marked by * were statistically significant ($P<0.05$)

The mean thiocyanate content of smokers was compared in the six sites using one-way ANOVA. There was a significant difference between Ruiru and Kahawa Sukari, Ruiru

and Kiwanja (P values of 0.01 and 0.02 respectively) implying that most smokers were found to feed on cassava, cabbage and sorghum which have been reported to have cyanogenic compounds (Padmaja, 1995). Some practiced dairy farming, hence consumed cow's milk which has also been reported to have high SCN levels (Borger's and Junge, 1979). Apart from these foods, cigarette smoke inhaled by the subjects could have contributed to these levels of SCN. Statistical analysis indicated that there was no significant difference between Ruiru and all other sites ($P > 0.05$). Ruiru was just used as a reference point; any other site would give the same result after post-ANOVA analysis.

The SCN mean levels in urine of smokers at Githurai were relatively high compared to other sites. This can be explained by the fact that it is a market place where most of cyanogenic foods are cheaply available. Subjects confessed mixing sorghum or cassava with maize to mill for flour and took cabbage for the better part of their meal as a major vegetable. Githurai is along the busy Thika high way with a high vehicle traffic density, constant exposure to vehicle fumes could have lead to high levels of SCN in the body.

Most residents in Kahawa Wendani do their shopping in Githurai (the nearest market place). Therefore no statistically significant difference was observed between the two places since people live almost the same life style. However, Kahawa Sukari neighboring Kahawa Wendani had relatively low value, this can be attributed to different social class. Most subjects did not consume cyanogenic foods and preferred packed maize flour other than mixing with other cereal flour. Kahawa Sukari is fairly removed from the main Thika highway by about 51-100 meters; hence the subjects were not directly exposed to vehicle fumes except for cases of traveling or inhale since most vehicle fumes do diffuse far distances in air. Most subjects in this area smoked for pleasure when drinking. Few

exceptional subjects reported cases of addiction; all these factors can explain the low levels obtained in Kahawa Sukari.

The Lower mean thiocyanate levels obtained from Kenyatta University can be attributed to a relatively clean environment. There are no vehicle fumes to which the subjects were exposed in the compound however subjects were free to move. Assessing their diet, it was found out that most subjects barely consumed indigenous foods.

Kiwanja smokers and non-smokers had lower thiocyanate levels of 3.21± 1.16 mg/L and 2.14±1.38 mg/L respectively than those in other sites. The significant difference ($P < 0.05$) among smokers between Ruiru and Kiwanja is possibly due to the fact that; there is minimal use of firewood for cooking and consumption of cyanogenic foods often emit hydrogen cyanide.

The levels of SCN obtained in this study do not exceeded the lethal value of 10 mg/L (Ryan and Terry, 1997). However, accumulation of thiocyanate in the body may lead to mild or moderate cyanide poisoning (ATSDR, 1997).

4.3 Summary of respondents from the questionnaire

A questionnaire was used to investigate certain factors that may influence the levels of thiocyanate in the body. Firstly environmental exposure was considered in terms of occupational exposure, disposal of synthetic polymers and cooking fuel. Secondly, exposure to cigarette smoke was also investigated with respect to number of cigarette smoked per day, duration of smoking and exposure to environmental (second hand smoke) cigarette smoke. Lastly, diet of the subjects was also established by determining

the most frequently consumed food. The results are discussed in the following subsections.

4.3.1 Influence of HCN on passive smokers

Mean SCN levels (mg/L±SE) of exposed and non-exposed non-smokers to second hand smoke were compared. The results of comparison are shown in Table 4.2

Table 4.2: SCN levels in urine of non-smokers who were either exposed or not exposed to second hand smoke

Non-smoker	Mean±SE (mg/L)	N	Percentage	T calc	T crit	df	Mean diff	P value
Exposed	2.20±0.15	83	67.68	2.42	1.98	121	0.63	0.017
Not exposed	1.99±0.12	40	32.52					

From the questionnaire, non-smokers (n = 83) (67.68%) of various sampling sites reported to have been exposed to cigarette smoke from either members of the family who smoke or the vicinity. There was a significant difference between non-smokers exposed and those not-exposed (P; 0.017). This is a clear indication that cigarette smoke has an effect on the SCN content in the body. Earlier research has indicated that approximately one non-smoker dies due to second-hand smoke exposure for every eight smokers dying of smoking related diseases (Schick and Glantz, 2006). Other studies have indicated that non-smokers exposed to passive smoke absorb an equivalent of 0.1-1.0 cigarettes per day (Sherman, 1991).

4.3.2 Influence of number of cigarettes smoked per day

Mean SCN levels (mg/L±SE) among smokers was compared irrespective of the sampling site but considered the number of cigarettes smoked per day. The results of comparison are shown in Table 4.3.

Table 4.3: SCN levels in urine of smokers from study area with respect to number of cigarette smoked

No of Cigarette	Classification	N	Percentage	Mean ± SE (mg/L ± SE)	Range	P value*
1	Light smokers	15	11.72	2.68±0.32*	1.05 – 5.35	<0.05
2-5	Light smokers	60	46.88	3.27±0.19*	0.88 – 7.85	<0.05
6-10	Moderate smokers	34	26.56	4.16±0.23*	1.10 – 8.93	<0.05
>11	Heavy smokers	19	14.84	6.33±0.52*	2.78– 11.95	<0.05

(*) means that the mean thiocyanate levels were statistically significant (P<0.05)

There was a significant difference in the levels of SCN between subjects who smoked >11 and 1 cig/day, >11 and 2-5 cig/day, >11 and 6-10 cig/day since P value was less than 0.05 in all the cases. Earlier research carried out by Prue and Martin (1980) indicated a mean SCN content of 413 ± 172 mg/L in saliva. Even though the biological fluids are not similar, the reported values were significantly high (P<0.05), he therefore concluded that SCN concentration reliably reflects the rate of cigarette smoking (Scherer, 2006). Considering other earlier studies on coefficients of correlation between SCN in the body fluids and the daily cigarette consumption from a selection of studies compiled, daily cigarette consumption was found to have a statistically significant influence (Vogt *et al*., 1979).

4.3.3 Duration of smoking

Mean SCN levels (mg/L±SE) of smokers with respect to the period of smoking were compared. The results are shown in Table 4.4.

Table 4.4: SCN levels in urine samples of smokers with respect to period of smoking

Period of smoking	N	Percentage (%)	Mean SCN±SE(mg/L)	P value
<5 years	64	27.3	3.17±0.20	0.00
5-20 years	53	33.4	4.69±0.28	0.00
>20 years	11	6.4	4.31±0.51	0.14

A statistical significant difference between those who smoked less than 5 yrs and 5-20 yrs since the P value was 0.00. This can be attributed to the fact that those who had smoked for longer period (5-20 yrs) took more cigarettes per day and had thiocyanate accumulate in the body unlike those who had smoked for less than 5 yrs. However, there was no significant difference noted between those who had smoked for less than 5 yrs and those who had smoked for more than 20 yrs. This can be accounted for by the fact that those who smoked for more than 20 yrs were light smokers hence low value depicted. It has been established from earlier studies that the variation of SCN in the body fluid is influenced by the individual's smoking profile (puffing and inhalation profile) (Vesey *et al*., 1982). Similarly there was no significant difference between those who smoked less than five years and those who didn't smoke at all. This can be explained by the fact than smokers of less than 5 years were not addicts and smoked only on social gathering in bars.

4.3.4 Influence of diet on urine SCN levels

Mean SCN levels (mg/L±SE) of the subjects considering food consumption were analysed using t-test and one way ANOVA. Table 4.5 shown compares thiocyanate among cassava, sorghum and maize consumers and non-consumers who are either smokers or non-smokers.

Table 4.5: SCN levels in urine of smokers and non-smokers who are either consumers or non consumers of cassava, sorghum and maize

Food	Description				T calc	P^a value	df	P^b value
Cassava	Consumer mean SCN±SE(mg/L)	N	Non-consumer mean SCN±SE(mg/L)	N				0.84
Smoker	5.33 ± 0.49	20	3.63 ± 0.17	108	3.802	<0.05	126	
Non-smoker	3.18 ± 0.35	24	1.70 ±0.11	99	5.16	<0.05	121	
Sorghum								0.84
Smoker	3.95 ± 0.37	17	3.89 ± 0.19	111	0.12	0.90	126	
Non-smoker	3.32 ± 0.34	8	1.90 ± 0.13	115	2.88	0.01	121	
Maize								0.00
Smoker	3.46 ± 0.23	60	4.28 ± 0.24	68	2.45	0.02	126	
Non-smoker	1.66 ± 0.13	62	2.33 ± 0.20	61	2.73	0.01	104	

P^a value for t-test and P^b value for one way ANOVA

There was significant difference between smokers who consume cassava and non-consumers. There was a significant difference between non-smoker, cassava consumer and non-cassava consumer with those who consumed having a higher mean. This can be attributed to the fact that cassava is a cyanogenic food. The tubers for the bitter species have been reported to contain 380 mg/kg cyanide concentration (Honing *et al*., 1983).

These results were in good agreement with results reported by Okafor (2004), who carried out a research to determine cyanide exposure among cassava consumers and cigarette smokers. He reported mean SCN levels of 3.57 ± 0.60 mg/L and 13.99 ± 13.81 mg/L for cassava consumers and cigarette smokers respectively. The value obtained from our study for non-smoker, non- cassava consumer is slightly lower than the reported value. This could be because cassava foods are not considered to be staple meals in study areas. Again, the SCN value of cigarette smokers who were non-cassava consumers was lower than the one reported. This can be attributed to variation in parameters affecting smoking dose like number of cigarettes, brand and number of puffs per cigarette (Scherer, 2006).

Smokers who were sorghum consumers had relatively higher value of 3.95 ± 0.37 mg/L than non-consumers meaning that sorghum contributed to SCN content in the body of a smoker since sorghum plant have been reported to contain 2400 mg/kg cyanide concentration (Honing *et al.,* 1983). However, there was no significant difference between consumers and non-consumers since both cigarette smoke and sorghum contributes to cyanide levels in the body. There was a significant difference between non-smoker sorghum consumer and non-consumer. This clearly indicates that sorghum had made a positive contribution to thiocyanate content in the body (Honing *et al.,* 1983).

Maize is a stable food in Kenya and a majority of subjects reported to have used it as a major bulk in their diet. Statistical analysis indicated a significant difference ($P = 0.02$). The maize non-consumers but smokers indicated a higher value than smokers who consumed maize. This indicates that the difference could be due to other environmental factors and cigarette smoke rather than maize. There was also a significant difference

noted between non-smokers maize consumers and non-consumers (P = 0.007). Again the non-smokers, non-maize consumers had higher value than non-smokers but maize consumers. This means that the contribution to SCN levels could be due to environmental factors; second-hand tobacco smoke and fumes from incompletely burned synthetic polymers rather than maize.

Cassava was compared with other foods that were consumed. It was found out that there was no statistically significant difference between cassava and sorghum using one-way ANOVA since P > 0.05. However, there was a significant difference between cassava and maize, cassava and other foods (other than sorghum and maize) since the P value were less than 0.05. This confirms that sorghum is a cyanogenic food unlike maize (Sklarew and Hayes, 1984).

4.3.5 Influence of disposal of synthetic polymers

Mean SCN levels (mg/L ± SE) of smokers and non-smokers were compared with respective ways of disposing synthetic polymers. The results of comparison are shown in Table 4.6.

Table 4.6: SCN levels in urine of subjects from study areas with respect to disposal of synthetic polymers

Method	Smokers				Non-smokers				P values
	N	%	Mean±SE (mg/L)	Range (mg/L)	N	%	Mean±SE (mg/L)	Range (mg/L)	
Burn	51	39.84	3.89±0.29	1.05-9.99	45	36.59	2.24±0.20	0.07-6.70	0.13
Municipal waste	70	54.69	3.84±0.22	0.88-11.95	78	63.41	1.84±0.15	0.03-6.75	0.04
Recycle	7	5.47	4.56±0.78	1.95-7.49	0	0	0	0	0.04

Most of the household items are made of plastic, besides, packing of bought items from most shops are done in plastic bags. These bags together with broken plastic items are disposed in different ways. From the questionnaire used in this study, it was found out that a total of 96 subjects do burn their synthetic polymers.

It can be noted that those who collected their waste synthetic polymers for recycle showed higher levels than those who deposited their plastic waste to municipal wastes or burned. This could be due to heavy smoking since all who collected their wastes for recycling were smokers. There was a significant difference between those who collected the wastes for recycle and those who collected the waste to municipal disposal sites ($P = 0.04$). Considering table 4.6, the level of thiocyanate among the populations that burn their waste and those in urban areas (where municipal council collects waste) is comparable. This is due to the fact that municipal wastes are burned at damping sites. The level of thiocyanate among smokers who recycled (samples collected and changed from one form to one another) their waste was higher statistically (4.56 ± 0.78 mg/L) than that among those who burned and submitted waste to municipal council 3.89 ± 0.29 mg/L and 3.84 ± 0.22 mg/L respectively. This can be attributed to the fact that unlike burning which emits hydrogen cyanide, recycling of waste do not lower the concentration of thiocyanate but simply change them from one to another (Sklarew and Hayes, 1984).

4.3.6 Influence of period of stay in an area

Mean SCN levels (mg/L±SE) of smokers and non-smokers were compared with respect to the period one had stayed in the study area. The results of the comparison are shown in Table 4.7.

Table 4.7: SCN levels in urine of smokers from all study areas with respect to period of stay

Period	Smokers				Non-smokers				df	P value	T cri	T cal
	N	%	Mean±SE (mg/L)	Range (mg/L)	N	%	Mean±SE (mg/L)	Range (mg/L)				
1-4 yrs	60	46.88	3.29±0.18	0.88-7.24	47	38.21	1.49±0.17	0.03-4.79	105	0.04	1.98	6.98*
4-8 yrs	54	42.19	4.25±0.27	1.05-11.95	60	48.78	2.10±0.18	0.08-6.75	94	0.04	1.99	6.59*
>10 yrs	14	10.94	5.11±0.72	1.25-9.99	16	13.00	3.04±0.32	1.61-6.70	18	0.00	2.10	2.62*

(*) means that the mean thiocyanate levels were statistically significant (t-calc>t-crit)

Participants, n = 114 reported to have lived in their respective areas for 4-8 years. Of these, n = 60 (46.78%) were non-smokers and had a mean thiocyanate content of 2.10±0.18 mg/L in the range of 0.08-6.75 mg/L while 42.19% (n = 54) were smokers with a mean of 4.25±0.27 mg/L in the range of 1.05-11.95 mg/L. Comparison of these two mean values using t-test indicates a statistically significant difference (df; 94, t-crit; 1.99, t-calc; 6.59).

A total of 107 subjects lived in their respective areas for 1-4 years. Only 38.21% (n = 47) were non-smokers with a mean thiocyanate content of 1.49±0.17 mg/L in the range of 0.03-4.79 mg/L. A total of 60 (46.88%) subjects were smokers with mean SCN level of 3.29±0.18 mg/L in the range of 0.88-7.24 mg/L. Statistical analysis of these two using t-test indicates that there is a significance difference between smokers and non-smokers who had lived for 1-4 years (df; 105, t-crit; 1.98, t-calc; 6.98).

The least group lived in their areas for >10 years. Only 16 (13.00%) were non-smokers with mean thiocyanate of 3.04±0.32 mg/L in the range of 1.61-6.70 mg/L. There were 14 (10.94%) smokers who had stayed for more than 10 years and had mean SCN of 5.11±0.72 mg/L in the range of 1.25-9.99 mg/L. Using t-test, there was a significant difference between SCN levels smokers and non-smokers who had lived for this period (df; 18, t-crit; 2.10, t-calc; 2.61).

Using one- way ANOVA, it was noted that there was a significant difference between those who lived for 1-4 years and 4-8 years, 1-4 years and >10 years. It was noted that those who lived longer (>10 yrs) in an area had higher values than those who lived for a

short time (1-4 yrs). Longer stay means longer exposure to environment factors that influence cyanide levels whose accumulation in the body could be fatal.

4.4 Comparison of picrate paper and Lundquist methods.

The results of 15 urine samples of smokers purposively selected were as given in Table 4.8. These samples were analysed using both methods in triplicate and the results compared.

Table 4.8: Results for picrate paper method and Lundquist method

	PICRATE PAPER METHOD	LUNDQUIST METHOD
Sample no.	Smoker	Smoker
KS15s	2.5±0.01	2.50±0.01
R3s	9.99±0.04	9.99±0.02
KU20s	2.41±0.02	2.32±0.00
R5s	8.52±0.02	8.51±0.02
KI16s	4.06±0.01	4.02±0.03
R3s	9.99±0.04	9.99±0.04
R7s	8.23±0.31	8.21±0.02
R4s	13.91±0.59	13.93±0.00
R12s	7.15±0.04	7.15±0.00
R18s	9.19±0.02	8.13±0.05
KU4s	5.52±0.02	5.52±0.00
G10s	5.39±0.00	5.38±0.00
G11s	6.32±0.01	6.32±0.00
KU1s	5.17±0.16	5.17±0.00
G2s	6.94±0.01	6.98±0.03

The relationship between picrate paper and Lundquist methods were analysed using correlation coefficient. This analysis was mainly to cross check the accuracy of picrate paper method. Table 4.9 shows the correlation results.

Table 4.9: Comparison of SCN levels for smokers using picrate paper and Lundquist method

T-Test: Paired Two Sample for Means	Smokers	Smokers
	Picrate paper method	*Lundquist method*
Mean (mg/L)	7.02	6.94
Variance	9.49	9.32
n	15.00	15.00
Pearson Correlation	0.998	
Hypothesized Mean Difference	0.00	
df	14.00	
t Stat	1.10	
P(T<=t) one-tail	0.15	
t Critical one-tail	1.76	
P(T<=t) two-tail	0.29	
t Critical two-tail	2.14	

From the analysis, it can be observed that on average the two methods do not differ at 95% confidence level since the calculated value of t = 1.10 and the critical t value is 2.14 for 14 degree of freedom. Hence the two methods are comparable (r = 0.998).

A graph was generated to illustrate this relationship. A regression line of the thiocyanate content obtained with the picrate paper method plotted against the thiocyanate content obtained with Lundquist method for 15 samples (smokers) is shown in Figure 4.1.

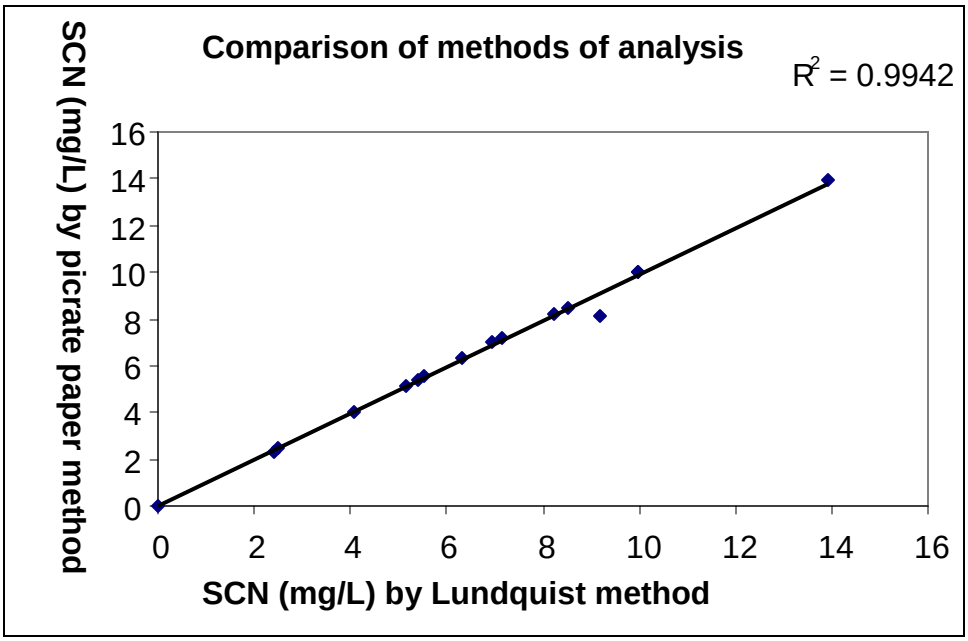

Figure 4.1 A plot of the results of the picrate paper method and Lundquist method

As can be seen from Figure 4.1, there was a very good correlation between the results from both methods with $r^2 = 0.9942$. Therefore, any of these two methods could be used since the correlation coefficient was close to one. In this study, picrate paper method was of choice because it is simple and requires cheaply available chemicals and equipment.

Study results obtained on SCN level was compared with some results obtained from other studies in the literature with similar background. The mean concentration of urine SCN in non-smoking subjects as determined by Lundquist method was 3.11 ± 4.23 mg/L which was higher compared to values published by Lundquist *et al,* 1995. Lundquist reported values of 0.043 ± 0.022mg/ L. The mean concentration of SCN for the 15 active smokers using the same method was 6.94 ± 9.32 mg/L. These values are higher than values previously reported by Lundquist *et al*.1995. His value was 0.057 ± 0.00 mg/L. It is evident that the environment in the study area is polluted with vehicle fumes, second

hand tobacco fumes, firewood smoke, fumes from burning synthetic polymers. The presence of SCN among non-smoker could be attributed to consumption of cyanogenic foods, exposure to environmental tobacco smoke, exposure to automobile exhaust fume fumes (ATSDR, 1997).

4.5 Comparison of chromatographic paper and Whatman 3 MM paper

A comparison of the SCN concentration of picrate paper method using picrate papers made from Whatman 3 MM and grade one cellulose chromatographic paper was done. The table 4.10 shows the results.

Table 4.10: SCN concentration of eight standards using Whatman 3 MM paper and chromatographic paper

Concentration in ppm	Whatmann 3 MM Paper	Chromatographic paper
0	0.00	0.00
1	0.117	0.051
2	0.197	0.195
3	0.295	0.241
4	0.372	0.349
5	0.468	0.334
6	0.550	0.379
7	0.646	0.494
8	0.783	0.642

The relationship between the SCN levels using these two papers were analysed using correlation coefficient. A regression line of the thiocyanate content obtained with the chromatographic paper plotted against the thiocyanate content obtained with Whatman 3MM paper for 8 standards whose concentration range from 0-100 mg/L is shown in Figure 4.2.

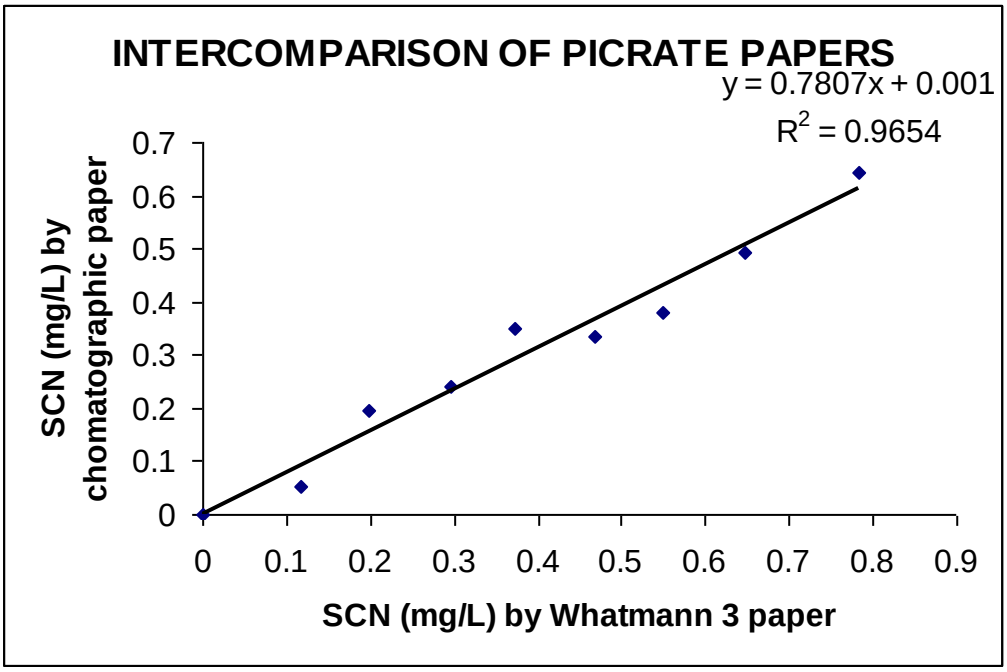

Figure 4.2 A plot of SCN content of the picrate paper method using chromatographic paper and Whatman 3 MM paper

It is evident from Figure 4.2 that there was a very good correlation between the results from both picrate papers with r^2 = 0.9654. It is therefore evident that grade one chromatographic paper can be used in place of Whatman 3 MM incase of scarcity since the correlation coefficient was close to one. This is because chromatographic paper is cheaply available.

4.6 Comparison of thiocyanate content from color chart and UV-visible spectrophotometer

There was need to compare the amount of thiocyanate obtained from the colour chart (visual comparison) with that obtained from direct spectrometric method. The relationship between the SCN levels of the two measurements was analysed using correlation coefficient. A regression line of the thiocyanate content obtained from colour chart comparison values plotted against the thiocyanate content obtained with UV-

visible spectrophotometer for 10 standards whose concentration range from 0-100 mg/L is shown in Figure 4.3.

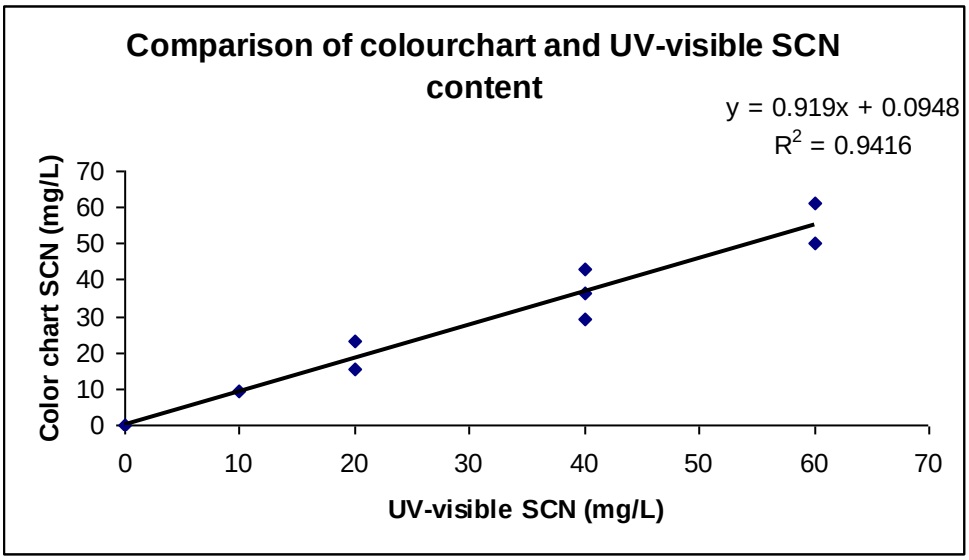

Figure 4.3 A plot of SCN content of the colourchart and UV-visible spectrophotometer measurements

There was a very good correlation between the results from both determinations with r^2 = 0.9416. Therefore, the colour chart can be used for quick analysis as a guide in field work to give thiocyanate content in samples especially where UV-visible spectrophotometer is not available since the correlation coefficient was close to one

4.7 Conclusion

With respect to the results obtained from this study, the following conclusions can be made:-

i. The concentration of thiocyanate in urine of smokers was significantly higher than thiocyanate content for non-smokers in all the study area.

ii. The periods of smoking as well as the number of cigarettes per day were found to have a significant influence on the amount of thiocyanate in the body.

iii. Certain factors have an influence on the levels of SCN in the urine of subjects. Factors identified for influencing SCN levels include, number of cigarettes smoked per day and diet (consumption of cyanogenic foods like cassava, sorghum).

4.8 Recommendations

4.8.1 Recommendations from this study

Presence of SCN in urine of smokers and non-smokers indicate the significant levels in the environment. Strategies to protect the general public like eliminating environmental sources should be put in place. Specific recommendations of this study are:-

i. Encourage policy makers not only to ban public smoking but also practice public campaigns and awareness on toxic compounds other than cigarette smoke.

ii. Sensitize people on indigenous food consumption as well as smoke and fumes in our environment that can increase cyanide levels in our bodies.

iii. Since there no significant difference between the methods explored, they can be used interchangeably as facilities may allow.

4.8.2 Recommendations for further study

These are further studies recommended to assess risks associated with cyanide in the body:-

i. Studies on assessment of thiocyanate content in children living with smokers in relation to their diet.
ii. Studies to establish the effect of other drugs like alcohol on thiocyanate content in the body.
iii. Studies to determine the amount of HCN in air along the busy highways and in smoking zones.
iv. Studies to determine urine SCN reference levels.
v. Study to be carried out among non-smokers who live with smokers, compare SCN levels of non-smokers who live in ventilated houses and those who don't.
vi. Levels of SCN among communities whose major stable food is cassava.
vii. Studies to determine the amount of thiocyanate among women who use various fuel for cooking

REFERENCES

Agency for Toxic Substances and Diseases Registry (ATSDR) (2004). *Toxicological profile for cyanide*. Atlanta, GA, US Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, pp 152-154.

Agency for Toxic Substances and Diseases Registry (ATSDR) (1997). *Toxicological profile for cyanide*. Atlanta, GA, US Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, pp 30865-30868.

Agency for Toxic Substances and Disease Registry (ATSDR) (1989). *Toxicological Profile for Cyanide*. ATSDR/TP-88/12; PB90-162058. Prepared by Syracuse Research Corporation for ATSDR, U.S. Public Health Service, under Contract No. 68-C8-0004.

Agrawal, V., Cherian, L. and Gupta, V.K. (1991). Extraction spectrophotometric method for the determination of hydrogen cyanide in environmental samples using 4-aminosalicylic acid. *International Journal of Environmental Analytical Chemistry*, **45**:235-244.

Andrew, S. (1985). Introduction to organic chemistry. 3rd ed. Macmillan publishing company, New York, pp 832-833.

Ansell, M and Lewis, F.A.S. (1970). A review of cyanide concentrations found in human organs- a survey of literature concerning cyanide metabolism,"normal,"nonfatal, and fatal body cyanide levels. *Journal of Forensic Medicine*, **17**:148-151.

Assaf, A.R., Parker, D., Lapane, K.L., McKenney, J.L. and Carleton, R.A. (2002). Are there gender differences in self-reported smoking practices? Correlation with thiocyanate and cotinine levels in smokers and nonsmokers from the Pawtucket Heart Health Program. *Journal of Womens Health,* **11**:899-906.

Banea, M., Poulter, N.H. and Rosling, H. (1992). Shortcuts in cassava processing and risk in diatary cyanide exposure in Zaire. Food Nutrition, pp 137-143.

Baumeister, G.H., Schievelbein, H. and Zickgraf, G. (1975). Toxicological and clinical aspects of cyanide metabolism. *Drug Respiratory*, **25**:1056-1064.

Borgers, D. and Junge, B. (1979). Thiocyanate as an indicator of tobacco smoking. *Preview Medicine*, **8**:351-357.

Bottoms, S.F., Judge, N.E., Kushner, P.M. and Sokol, R.J. (1982) Thiocyanate and drinking during pregnancy. Alcoholism. *Clinical Experimental Research*, **6**:391-395.

Bhide, S.V. and Jayant, K. (1987). Salivary thiocyanate levels and biological marker in tobacco habitues: a study on Indian sample. *Tobacco Respiration*, **13**:9-13.

Bliss, R.E. and O'Connell, K.A. (1984). Problems with thiocyanate as an index of smoking status: a critical review with suggestions for improving the usefulness of biochemical measures in smoking cessation research. *Health Psychology*, **3**:563-581.

Bradbury, M.G., Egan, S.V. and Bradbury, J. H. (1999). Determination of all forms of cyanogens in cassava roots and cassava products using picrate papers kits. *Journal of the Science of Food and Agriculture*, **79**:593-601.

Butts, W.C., Kuehneman, M. and Widdowson, G.M. (1974). Automated method for determining serum thiocyanate, to distinguish smokers from nonsmokers. *Clinical Chemistry*, **20**:1344-1348.

Carotti, A. A and Kaiser, E.R. (1972). Concentrations of twenty gaseous chemical species in the flue gas of a municipal incinerator. *Journal of the Air Pollution Control Association*, **22**:224-253.

Central Bureau of Statistics (CBS),(2000). Kenya population census 1999, ministry of planning and development, Nairobi Kenya.

Chandra, H., Gupta, B.N., Bhargava, S.K., Clerk, S.H. and Mahendre, P.N. (1980). Chronic cyanide exposure: a biochemical and industrial hygiene study. *Journal of Analytical Toxicology*, **4**:161-165

Chinaka, S., Takayama, N., Michigama, Y. and Uneda, K. (1998). Simultaneous determination of cyanide and thiocyanate in blood by ion chromatography with fluorescence and ultraviolet detection. *Journal of Chromatography B*, **713**:353-359.

Clark, C. J., Campbell, D. and Reid, W.H. (1981). Blood carboxyhaemoglobin and cyanide levels in fire survivors. *Lancet*, **i**:1332-1335.

Cohen, J.D. and Bartsch, G.E. (1980) A comparison between carboxyhemoglobin and serum thiocyanate determinations as indicators of cigarette smoking. *American Journal of Public Health*, **70**:284-286.

Cohen, D.J., Michel, D., Donald, E. O., Kalon, K.L., Jeffrey, J.P. and Richard, E. K. (2001)."Impact of smoking on clinical and angiographic restenosis after percutaneous coronary intervention"circulation. *British Medical Journal*, **104**:773-778.

Cock, J. H. (1985). Cassava: New potential for neglected crop. West view press, Boulder, CO, USA, pp10-11.

Cooke, R.D., Richard, J.E. and Thompson, A.R. (1985) Nutritional aspect of cassava storage and processing.7th Symposium of the international society for tropical root crops, pp 645-648.

Dalhamm, T. (1970). In vivo and in vitro ciliotoxic effects of tobacco smoke. *Archives Environmental Health*, **21**:633-634.

Daniel, W.W. (1999). Biostatistics: A Foundation for analysis in the health sciences. 7th ed. New York: John Wiley and Sons, pp 9-12.

Degiampietro, P., Peheim, E., Drew, D., Graf, H. and Colombo, J.P. (1987). Determination of thiocyanate in plasma and saliva without deproteinisation and its validation as a smoking parameter. *Journal of Clinical Chemistry Clinical Biochemistry*, **25**:711-717.

Delenge, F., Ekpechi, L. O. and Rosling, H. (1994). Cassava cyanogenesis and iodine deficiency disorder. *Acta Horticulturae,* **375**:280-293.

Densen, P.M., Davidow, B., Bass, H.E. and Jones, E.W. (1967) A chemical test for smoking exposure. *Archives of Environmental Health*, **14**:865-874.

DFG-German Science Foundation. (1985). Analyses of hazardous substances in biological materials, 1st ed. Weinheim: VCH Verlagsgesellschaft mbH.

Doll, R and Hill, A. B. (1950). "Smoking and carcinoma of the lung. Preliminary report". *British Medical Journal*, **2**:739-748.

Doll, R., Peto, R., Boreham, J. and Sutherland, L. (2004). "Mortality in relation to smoking: 50 years 'observations' on male." *British Medical Journal*, **326**:1519-1527.

ECETOC (2004) *Hydrogen cyanide, sodium and potassium cyanides and acetone cyanohydrin*. Brussels, European Centre for Ecotoxicology and Toxicology of Chemicals, in press (ECETOC Joint Assessment of Commodity Chemicals).

Egan, S.V., Yeoh, H.H. and Bradbury, J.H. (1998). Simple picrate paper kit for determination of the Cyanogenic potential of cassava flour. *Journal of science food and agriculture*, **76**:39-48.

Ellenhorn, M. J and Barceloux, D.G. (1988). Medical toxicology. Diagnosis and treatment of human poisoning. Elsevier Science Publishing Company, New York, pp 1477-1479.

Ermans, A. M., Delange, F., Van Der Velden, M. and Kinthaert, S. (1972). Possible role of cyanide and thiocyanate in the etiology of endemic cretinism. *Advances in Experimental Medicine and Biology*, **30**:455-486.

Ermans, A.M., Mbulamoko, N.M., Delange, F. and Ahluwalia, R. (1980). Role of cassava in the etiology of endemic goitre and cretinism. Ottawa, Ontario, International Development Research Centre, pp 182-185.

Fiksel ,J., Cooper, C., Eschenroeder, A., Goyer, M. and Perwak, J. (1981). Exposure and risk assessment for cyanide. Washington, DC, US Environmental Protection Agency (EPA/440/4-85/008; NTIS PB85-220572).

Feldstein, M and Klendshoj, N.C. (1954). The determination of cyanide in biological fluids by microdiffusion analysis. *Journal of Laboratory and Clinical Medicine*, **44**:166-170.

Fortmann, S.P., Rogers, T., Vranizan, K., Haskell, W.L., Solomon, D.S. and Farquhar, J.W. (1984). Indirect measures of cigarette use: expired-air carbon monoxide versus plasma thiocyanate. *Preview Medicine*, **13**:127-135.

Foster, S. E., Jones, D. J., Olson, A. L., Forehand, R. and Bau, J. J. (2007). Family socialization of Adolescent's self reported cigarette use: the role of parent's history of regular smoking and parenting style. *Journal pedriatrics psychology* **32**:481-493.

Frakes, R.A., Sharma, R.P. and Willhite, C.C. (1986a). Metabolism of linamarin and amygdalin in hamsters. *Food and Chemical Toxicology*, **24**:417-420.

Gettler, A. O and Baine, J. O. (1938). The toxicology of cyanide. *American Journal of Medical Science,* **195**:138-141.

Gillilang, F.D., Islam, T., Berhanek, M.C., Gauderman, W. J., Connell, R., Avol, E. and Peter, J. M. (2006). Regular smoking and asthma incidence in adolescents. *American Journal of Respiratory critical care and medicine,* **174**:1094-10100.

Gosselin, R.E., Smith, R.P. and Hodge, H .C. (1984*). Clinical toxicology of commercial products* .5th edition. Williams and Wilkins,Baltimore, pp 111-126.

Guerin, M.R. (1980). Chemical composition of cigarette smoke. Banbury report. A safe cigarette? Cold spring harbour laboratory, New York, pp 191-204.

Green, J.T., Richardson, C., Marshall, R.W., Rhodes, J., McKirdy, H.C., Thomas, G.A. and Williams, G.T. (2000). "Nitric oxide mediates a therapeutic effect of nicotine in ulcerative colitis". *Aliment Pharmacological Ther*apy, **14**:1429-1434.

Haley, N.J., Axelrad, C.M. and Tilton, K.A. (1983). Validation of self-reported smoking behaviour: Biochemical analyses of cotinine and thiocyanate. *American Journal of Public Health,* **73**:1204-1207.

Haque, R and Bradbury, J. H. (1999). Simple kit method for determination of thiocyanate in urine. *Clinical chemistry*. **45**:1459-1464.

Hartung, R. (1982). "Cyanides and nitriles." In: *Patty's Industrial Hygiene and Toxicology*, vol. 2C, G.D. Clayton and E. Clayton, eds. John Wiley & Sons, New York, NY, pp 4845-4900.

Henningfield, J. E and Griffiths, R. R. (1981) Cigarette smoking and subjective response: Effects of d-amphetamine. *Clinical Pharmacolological Therapy*, **30**:497-505.

Hoffmann, D., Hoffmann, I. and El-Bayoumy, K. (2001). The Less Harmful Cigarette: A Controversial Issue. A tribute to Ernst L. Wynder. *Chemical Research in Toxicology,* **14**: 767-790.

Hoffmann, D and Hoffmann, I. (1997). The changing cigarette, 1950-1995. *Journal of Toxicological Environmental Health,* **50**:307-364

Holland, D. J. (1983). Cyanide poisoning an uncommon encounter. *Journal of Emergency Nursing,* **9**:138-141.

Honig, D.H., Hockridge, M.E., Gould, R.M. and Rackis, J.J. (1983) Determination of cyanide in soybeans and soybean products. *Journal of Agricultural and Food Chemistry*, **31**:272-275.

Howard, J.W. and Hanzal, R.F. (1955). Chronic toxicity to rats of food treated with hydrogen cyanide. *Journal of Agricultural and Food Chemistry,* **3**:325-329.

International Agency for Research on cancer (IARC), (1986). Tobacco smoking IARC monograraphs on the evaluation of the carcinogenic risk of chemicals to humans, 38th ed.

Jarvis, M.J., Tunstall-Pedoe, H., Feyerabend, C., Vesey, C. and Saloojee, Y. (1984b). Biochemical markers of smoke absorption and selfreported exposure to passive smoking. *Journal of Epidemiologic Commune Health,* **38**:335-339.

Karuri, E.E., Mbugua, S. K., Karugia, J., Wanda, K, and Jangwe, J. (2001) An assessment of the industrial potential in Kenya, Market study, foodnet, Kampala, Uganda.

Kirk-Othmer. (1979).Encyclopedia of chemical technology Vol 7,.3rd edition, John Wiley and sons, New York, pp 308-310.

Korpilahde, T., Heliovaara, M., Knekt, P., Marniemi, J., Aromaa, A. and Aho, K. (2004). Smoking history and serum cotinine and thiocyanate concentrations as determinants of rheumatoid factor in non-rheumatoid subjects. *Rheumatology,* **43**:1424-1428.

Landahl, H.D. and Herrmann, R.G. (1950). Retention of vapours and gases in the human nose and lung. *Archives of Industrial Hygiene and Occupational Medicine*, **1**:36-45.

Lauwerys, R.R. and Hoet, P. (2001). Industrial chemical exposure. Guidelines for biological monitoring, 3rd ed. Boca Raton, FL, Lewis Publishers.

Leuschner, J., Winkler, A. and Leuschner, F. (1991). Toxicokinetic aspects of chronic cyanide exposure in the rat. *Toxicology Letter*. **57**:195-201.

Luepker, R.V., Pechacek, T.F., Murray, D.M., Johnson, C.A., Hund, F. and Jacobs, D.R. (1981). Saliva thiocyanate: a chemical indicator of cigarette smoking in adolescents. *American Journal of Public Health*, **71**:1320-1324.

Lundquist, P., Kagedal, B. and Nilsson, L. (1995). An improved method for determining thiocyanate in plasma and urine. *European journal of Clinical Chemistry clinical biochemistry*, **33**:343-349.

Li, Q., Jacob, D.J., Bey, I., Yantosch, R.M., Zhao, Y., Kondo, Y. and Notholt, J. (2000). Atmospheric hydrogen cyanide (HCN): Biomass burning source, ocean sink? *Geophysical Research Letters*, **27**:357-360.

Michigami, Y., Fujii, K., Ueda, K. and Yamamoto, Y. (1992). Determination of thiocyanate in human saliva and urine by ion chromatography. *Analyst*, **117**:1855-1888.

Mucklow, J.C., Bending, M.R., Kahn, G.C., Dollery, C.T. (1978) Drug concentration in saliva. *Clinical Pharmacological Therapy*, **24**:563-570.

Muranaka, H., Higashi, E., Itani, S. and Shimizu, Y. (1988). Evaluation of nicotine, cotinine, thiocyanate, carboxyhemoglobin, and expired carbon monoxide as biochemical tobacco smoke uptake parameters. *International Archive Occupational Environmental Health,* **60**:37-41.

National Coordination Agency for Population and Development (NCAPD) (2005). Thika District Strategic Plan 2005-2010 for Implementation of the National Population Policy for Sustainable Development. HSK Consulting Ltd. pp 3-4.

National Institute for Occupational Safety and Health (NIOSH) (1976). *Occupational Exposure to Hydrogen Cyanide and Cyanide Salts*. U.S. Department of Education and Welfare, Public Health Service, Rockville, MD. Publ. No. 1-191.

National Institute of health (NIH) (1998). Cigars; health effects and trends. Washington DC, US department of health and human services, pp 232.

Nikitin, Y.P., Shalaurova, I.Y. and Serova, N.V. (1990).The validation of serum thiocyanate smoking data in a population survey. *Rev E´ pide´ miol Sante´ Publ,* **38**:469-472.

Norris, J.C., Moore, S.J. and Hume, A.S. (1986).Synergistic lethality induced by the combination of carbon monoxide and cyanide, *Toxicology*, **40**:121-129

Ockene, J.K., Pechacek, T.F., Vogt, T. and Svendsen, K. (1987). Does switching from cigarettes to pipes of cigars reduce tobacco smoke exposure? *American Journal of Public Health*, **77**:1412-1416.

Okafor, P.N. (2004). Assessment of cyanide overload in cassava consuming populations of Nigeria and the cyanide content of some cassava based foods. *African journal of biotechnology*, **3**:358-361.

Osuntokun, B.O. (1994). Chronic cyanide intoxication of dietary origin and a degenerative neuropathy in Nigerians. *Acta Horticulturae*, **375**:311-321.

Padmaja, G. (1995). Cyanide detoxification in cassava for food and feed use. *Critical Reviews in Food Sciences and Nutrition*, **35**:259-339.

Pettersen, J.C. and Cohen, S.D. (1985). Antagonism of cyanide poisoning by chlorpromazine and sodium thiosulphate. *Toxicol Applied Pharmacology,* **81**:265-273.

Prignot, J. (1987). Quantification and chemical markers of tobaccoexposure. *European Journal of Respiratory Diseases,* **70**:1-7.

Prue, D.M., Martin, J.E., Hume, A.S. and Davis, N.S. (1981b). The reliability of thiocyanate measurement of smoking exposure. *Addiction Behaviour,* **6**:99-105.

Rickert, W.S., and Robinson, J.C. (1981) Estimating the hazards of less hazardous cigarettes. II. Study of cigarette yields of nicotine, carbon monoxide, and hydrogen cyanide in relation to levels of cotinine, carboxyhemoglobin, and thiocyanate in smokers. *Journal of Toxicology and Environmental Health*, **7**:391-403.

Roberts, D.L. (1988). Natural tobacco flavor. *Recent advanced tobacco,* **14**:45-81.

Rose, C.L., Worth, R.M., Kikuchi, K. and Chen, K.K. (1965). Cobalt salts in acute cyanide poisoning. *Proceedings of Society Experimental Biological Medicine,* **120**:780-783.

Ruth, K.J. and Neaton, J.D. (1991). Evaluation of two biological markers of tobacco exposure. *Preview Medicine,* **20**:574-589.

Ryan, R.P. and Terry, C.E (1997) toxicology desk reference 4th ed. Taylor and francis, Washington, D.C, pp 911-912.

Seto, Y., Tsunoda, H., Ohta, H and Shinohara, T. (1993). Determination of blood cyanide by headspace gas chromatography with nitrogen phosphorus detection and using a megabore capillary column. *Analytica Chemica Acta,* **276**:247-259.

Setting, M. (1985). Handbook of Toxic and hazardous chemicals and carcinogenic, 2nd ed. Park Ridge, NJ: Noyes Data Corporation, pp 508-509.

Scherer, G. (2006). Carboxyhemoglobin and thiocyanate as biomarkers of exposure to carbon monoxide and hydrogen cyanide in tobacco smoke. *Experimental and toxicologic pathology*, **58**:101-124.

Schick, S. and Glantz, S.A. (2006) Sidestream cigarette smoke toxicity increases with ageing and exposure duration. *Tobacco Control*, **15**:424-429.

Sherman, C.B. (1991) Health effects of cigarette smoking. *Clinical Chest Medicine*, **12:**643-658.

Silverman, S.H., Purdue, G.F., Hunt, J.L and Bost, R.O. (1988) Cyanide toxicity in burned patients, *Journal of Trauma*, **28**:171-176.

Solomonson, L. P. (1981). Cyanide as a metabolic inhibitor. In: Vennesland B *(*eds) Cyanide in biology. Academic press, London and New York, pp 11-28.

Sklarew, D.S. and Hayes, D.J. (1984). Trace nitrogen-containing species in the offgas from 2 oil shale retorting processes. *Environmental Science and Technology*, **18**:600-603.

Sylvester, D.M., Hayton, W.L., Morgan, R.L. and Way, J.L. (1983). Effects of thiosulfate on cyanide pharmacokinetics in dogs. *Toxicol applied pharmacology,* **69**:265-271.

Torano, J.S. and van Kan, H.J. (2003). Simultaneous determination of the tobacco smoke uptake parameters nicotine, cotinine and thiocyanate in urine, saliva and hair, using gas chromatography-mass spectrometry for characterisation of smoking status of recently exposed subjects. *Analyst*, **128**:838-843.

United States Environmental Protection Agency. (1987). Drinking water criteria document for cyanide. Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH, for the Office of Drinking Water, Washington, DC. External review draft. cited in reference 4.

United States Environmental Protection Agency (1983) Methods for chemical analysis of water and waste. Method 335.2. Cyanide, *total.* Cincinnati, OH, US Environmental Protection Agency, Environmental Monitoring and Support Laboratory

United States Enviromental Protection Agency (1985). *Drinking Water Criteria Document for Cyanide (Final Draft)*. Prepared by the Environmental Criteria and Assessment Office for the Office of Drinking Water,Cincinnati, OH. ECAO-CIN-442, PB86-117793.

Vesey, C.J., Saloojee, Y., Cole, P.V. and Russell, M.A.H. (1982). Blood carboxyhaemoglobin, plasma thiocyanate, and cigarette consumption: implications for epidemiological studies in smokers. *British Medicinal Journal*, **284**:1516-1518.

Vogt, T.M., Selvin, S. and Hulley, S.B. (1979) Comparison of biochemical and questionnaire estimates of tobacco exposure. *Preview Medicine*, **8**:23–33.

Walters, M.I. and Sawhney, A.K. (1987). A simple and rapid method for the determination of thiocyanate in serum. *Journal of Analytical Toxicology*, **11**:53-54.

Ware, G.W. (1989). United States Environmental Protection Agency Office of Drinking Water Health advisories. *Reviews in Enviromental Contamination Toxicology*, **2**: pp 107-109.

Way, J.L., Sylvester, D. and Morgan, R. L. (1984). Recent perspectives on the toxicodynamic basis of cyanide antagonism. *Fundamental applied toxicology,* **4**:231-239.

Westley, J. (1980). Rhodanese and the sulfane pool. In**:** *Enzymatic Basis of Detoxication*, Vol. II. Academic Press, New York, pp 245-262.

World Health Organization, (2004). Hydrogen cyanide and cyanides: Human health aspects, International Programme on Chemical Safety (IPCS), Concise International Chemical Assessment Document 61.

World Health Organization. (1984). Cyanide. In:Guidelines for drinking water. Vol. 2. Health criteria and other supporting information.Geneva pp. 97-98.

Yates, D.H., Breen, H. and Thomas,P.S. (2001). Passive smoke inhalation decreases exhaled nitric oxide in normal subjects. *American journal of respiratory critical care medicine,***164**:1043-1046.

APPENDIX I

Table A1: Individual raw data of mean SCN level in urine of smokers and non-smokers from study areas

Githurai		Ruiru		Kenyatta University		Kahawa Wendani		Kahawa Sukari		Kiwanja	
Sample	UV SCN (mg/L)±SD	Sample	UV SCN (mg/L)±SD	Sample	UV SCN (mg/L)±SD	Sample	UV SCN (mg/L)±SD	Sample	UV SCN (mg/L)±SD	Sample	UV SCN (mg/L)±SD
g10n	0.63±0.01	r10n	0.40±0.02	ku10n	2.91±0.03	kw10s	6.17±0.01	ks10n	1.65±0.07	ki10n	0.85±0.01
g10s	5.39±0.00	r10s	5.78±0.01	ku10s	3.22±0.05	kw11n	1.90±0.03	ks10s	3.74±0.00	ki10s	2.99±0.03
g11n	2.42±0.01	r11n	0.41±0.02	ku11n	2.79±0.01	kw11s	1.32±0.01	ks11n	4.14±0.00	ki11n	0.45±0.01
g11s	6.32±0.01	r11s	5.40±0.02	ku11s	4.08±0.05	kw12n	2.27±0.00	ks11s	3.35±0.00	ki11s	2.59±0.01
g12n	2.05±0.04	r12n	3.67±.0.01	ku12n	2.34±0.03	kw12s	3.29±0.01	ks12n	0.48±0.02	ki12n	1.93±0.05
g12s	3.96±0.083	r12s	7.15±0.04	ku12s	2.94±0.04	kw13n	2.97±0.01	ks12s	4.22±00	ki12s	2.19±0.00
g13n	2.67±0.03	r13n	2.73±0.01	ku13n	1.78±0.01	kw13s	5.46±0.01	ks13n	1.23±0.01	ki13n	2.36±0.00
g13s	5.37±0.04	r13s	5.62±0.00	ku13s	3.61±0.05	kw14n	2.35±0.02	ks13s	3.21±0.01	ki13s	2.17±0.00
g14n	1.25±0.00	r14n	3.54±0.07	ku14n	2.65±0.02	kw14s	4.11±0.02	ks14n	0.42±0.03	ki14n	4.82±0.00
g14s	1.90±0.06	r14s	2.42±0.01	ku14s	1.70±0.18	kw15n	1.79±0.00	ks14s	3.36±0.01	ki14s	5.39±0.00
g15n	4.30±0.01	r15n	3.20±0.01	ku15n	7.39±0.03	kw15s	4.05±0.02	ks15s	0.35±0.00	ki15n	2.64±0.00
g15s	2.40±0.04	r15s	1.36±0.00	ku15s	2.44±0.07	kw16n	2.83±0.00	ks15n	2.50±0.01	ki15s	3.75±0.00
g16n	4.76±0.01	r16n	4.06±0.08	ku16n	2.21±0.03	kw16s	3.45±0.01	ks16s	0.78±0.02	ki16n	3.41±0.01
g16s	6.34±0.02	r16s	1.10±0.01	ku16s	3.78±0.04	kw17n	1.97±0.00	ks16n	2.80±0.01	ki16s	4.06±0.01
g17n	1.25±0.01	r17n	2.64±0.04	ku17n	2.31±0.03	kw17s	1.73±0.01	ks17n	0.32±0.00	ki17n	2.55±0.00
g17s	3.23±0.17	r17s	4.84±0.00	ku17s	3.63±0.03	kw18n	3.06±0.00	ks17s	5.31±0.01	ki17s	3.44±0.00
g18s	5.30±0.26	r18n	1.95±0.01	ku18n	1.57±0.02	kw18s	1.41±0.02	ks18n	1.19±0.01	ki18n	2.80±0.00
g19s	3.52±0.01	r18s	9.19±0.02	ku18s	4.03±0.11	kw19n	0.39±0.00	ks18s	2.97±0.01	ki18s	1.49±0.01
g1n	7.49±0.04	r19n	0.86±0.00	ku19n	0.41±0.02	kw19s	3.43±0.01	ks19n	1.51±0.28	ki19n	3.00±0.05
g1s	6.32±0.02	r19s	4.55±0.00	ku19s	2.89±0.06	kw1n	1.56±0.02	ks19s	1.89±0.01	ki19s	1.72±0.00
g20n	2.06±0.03	r1n	3.68±0.01	ku1n	0.16±0.00	kw1s	6.79±0.01	ks1n	4.54±0.01	ki1n	4.92±0.01
g20s	2.27±0.01	r1s	3.09±0.03	ku1s	5.17±0.16	kw20n	2.40±0.00	ks1s	5.40±0.02	ki1s	4.91±0.01
g21n	2.13±0.02	r20n	0.35±0.03	ku20n	0.65±0.03	kw20s	1.11±0.01	ks20n	0.60±0.01	ki20n	2.17±0.00

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Githurai		Ruiru		Kenyatta University		Kahawa Wendani		Kahawa Sukari		Kiwanja	
Sample	UV SCN (mg/L)±SD	Sample	UV SCN (mg/L)±SD	Sample	UV SCN (mg/L)±SD	Sample	UV SCN (mg/L)±SD	Sample	UV SCN (mg/L)±SD	Sample	UV SCN (mg/L)±SD
g21n	2.13±0.02	r20n	0.35±0.03	ku20n	0.65±0.03	kw20s	1.11±0.01	ks20n	0.60±0.01	ki20n	2.17±0.00
g21s	4.74±0.03	r20s	3.81±0.02	ku20s	2.41±0.02	kw21s	3.52±0.01	ks20s	1.73±0.01	ki20s	2.99±0.03
g22n	3.06±0.33	r21n	2.10±0.01	ku22s	0.75±0.05	kw3n	2.36±0.02	ks2n	2.35±0.02	ki21n	1.92±0.04
g22s	1.16±0.01	r21s	4.59±0.00	ku2n	0.15±0.01	kw3s	4.36±0.00	ks2s	2.35±0.01	ki21s	4.92±0.01
g23s	3.43±0.01	r2n	0.62±0.02	ku2s	2.96±0.02	kw4n	4.10±0.03	ks3n	2.93±0.05	ki2n	4.45±0.01
g2n	0.48±0.02	r2s	1.21±0.12	ku3n	6.60±0.04	kw4s	7.66±0.01	ks3s	4.45±0.00	ki2s	4.63±0.02
g2s	6.94±0.01	r3n	3.06±0.01	ku3s	6.15±0.02	kw5n	2.18±0.00	ks4n	1.88±0.01	ki3n	0.16±0.01
g3n	1.65±0.01	r3s	9.99±0.04	ku4n	2.15±0.04	kw5s	3.43±0.00	ks4s	3.59±0.00	ki3s	3.05±0.09
g3s	5.14±0.01	r4n	0.95±0.01	ku4s	5.52±0.02	kw6n	3.29±0.01	ks5s	4.69±0.01	ki4n	0.40±0.01
g4n	2.67±0.03	r4s	13.91±0.59	ku5n	2.69±0.04	kw6s	4.01±0.01	ks6n	2.75±0.02	ki4s	1.58±0.02
g4s	5.08±0.01	r5n	0.63±0.01	ku5s	4.45±0.01	kw7n	1.02±0.01	ks6s	1.24±0.01	ki5n	2.34±0.01
g5n	0.86±0.00	r5s	8.52±0.02	ku6n	2.80±0.02	kw7s	3.44±0.01	ks7n	1.59±0.03	ki5s	4.92±0.01
g5s	4.57±0.02	r6n	3.04±0.01	ku6s	1.95±0.09	kw8n	0.71±0.01	ks7s	1.96±0.01	ki6n	1.72±0.00
g6n	3.35±0.00	r6s	7.04±0.05	ku7n	0.94±0.02	kw8s	3.35±0.00	ks8n	1.45±0.05	ki6s	3.81±0.02
g6s	4.39±0.02	r7n	0.55±0.00	ku7s	4.84±0.01	kw9n	1.80±0.00	ks8s	2.35±0.00	ki7n	1.25±0.00
g7n	0.32±0.00	r7s	8.23±0.31	ku8n	2.49±0.00	kw9s	4.82±0.28	ks9n	1.18±0.01	ki7s	4.14±0.01
g7s	2.26±0.03	r8n	0.39±0.01	ku8s	3.22±0.02	kw2s	7.19±0.02	ks9s	5.38±0.00	ki8n	2.18±0.00
g8n	1.17±0.00	r8s	2.89±0.15	ku9n	1.54±0.02	kw2n	2.41±0.01	ks22n	1.04±0.03	ki8s	4.15±0.01
g8s	3.36±0.36	r9n	1.57±0.01	ku9s	4.70±0.06			ks21n	0.07±0.00	ki9n	0.19±0.03
g9n	1.57±0.02	r9s	3.32±0.04	ku21s	3.89±0.02					ki9s	1.41±0.01
g9s	4.84±0.00			ku21n	0.79±0.02						

Key
g = Githurai: n = non- smoker: s = smoker
ki = Kiwanja: r = Ruiru.
ks = Kahawa Sukari: ku = Kenyatta University: kw = Kahawa Wendani

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Githurai		Ruiru		Kenyatta University		Kahawa Wendani		Kahawa Sukari		Kiwanja	
Sample	CC SCN (mg/L)±SD	Sample	CC SCN (mg/L)±SD	Sample	CC SCN (mg/L)±SD	Sample	CC SCN (mg/L)±SD	Sample	CC SCN (mg/L)±SD	Sample	CC SCN (mg/L)±SD
g10n	0.67±0.58	r10n	0.33±0.58	ku10n	2.67±1.15	kw10s	6.17±0.01	ks10n	1.67±0.58	ki10n	0.67±0.58
g10s	5.33±1.15	r10s	4.67±1.15	ku10s	2.67±1.15	kw11n	1.90±0.03	ks10s	3.33±1.15	ki10s	2.67±1.15
g11n	2.00±0.00	r11n	0.33±0.58	ku11n	3.33±1.15	kw11s	1.32±0.01	ks11n	4.00±0.00	ki11n	0.00±0.00
g11s	6.00±0.00	r11s	5.33±1.15	ku11s	3.33±1.15	kw12n	2.27±0.00	ks11s	2.00±0.00	ki11s	2.67±1.15
g12n	2.00±0.00	r12n	4.00±0.00	ku12n	2.00±0.00	kw12s	3.29±0.01	ks12n	0.00±0.00	ki12n	1.67±0.58
g12s	3.33±1.15	r12s	7.33±2.31	ku12s	4.00±0.00	kw13n	2.97±0.01	ks12s	3.33±1.15	ki12s	2.67±1.15
g13n	2.67±1.15	r13n	2.67±1.15	ku13n	1.33±058	kw13s	5.46±0.01	ks13n	1.00±0.00	ki13n	2.00±0.00
g13s	5.33±1.15	r13s	5.33±1.15	ku13s	3.33±1.15	kw14n	2.35±0.02	ks13s	3.33±1.15	ki13s	2.67±1.15
g14n	1.00±0.00	r14n	3.33±1.15	ku14n	2.67±1.15	kw14s	4.11±0.02	ks14n	0.67±0.58	ki14n	3.33±1.15
g14s	2.00±0.00	r14s	2.67±1.15	ku14s	1.67±0.58	kw15n	1.79±0.00	ks14s	3.33±1.15	ki14s	5.33±1.15
g15n	4.00±0.00	r15n	2.67±1.15	ku15n	6.00±0.00	kw15s	4.05±0.02	ks15s	0.33±0.58	ki15n	3.33±1.15
g15s	2.67±1.15	r15s	1.00±0.00	ku15s	2.00±0.00	kw16n	2.83±0.00	ks15n	3.33±1.15	ki15s	3.33±1.15
g16n	4.67±1.15	r16n	4.00±0.00	ku16n	2.00±0.00	kw16s	3.45±0.01	ks16s	0.67±0.58	ki16n	3.33±1.15
g16s	6.00±0.00	r16s	1.00±0.00	ku16s	4.00±0.00	kw17n	1.97±0.00	ks16n	2.67±1.15	ki16s	3.33±1.15
g17n	1.00±0.00	r17n	2.00±0.00	ku17n	2.00±0.00	kw17s	1.73±0.01	ks17n	0.33±0.58	ki17n	2.67±1.15
g17s	3.33±1.15	r17s	4.67±1.15	ku17s	3.33±1.15	kw18n	3.06±0.00	ks17s	5.33±1.15	ki17s	2.67±1.15
g18s	5.33±1.15	r18n	2.00±0.00	ku18n	1.33±0.58	kw18s	1.41±0.02	ks18n	1.00±0.00	ki18n	2.67±1.15
g19s	3.33±0.00	r18s	8.67±2.31	ku18s	4.00±0.00	kw19n	0.39±0.00	ks18s	2.67±1.15	ki18s	1.33±0.58
g1n	6.00±0.00	r19n	0.67±0.58	ku19n	0.33±0.58	kw19s	3.43±0.01	ks19n	1.00±0.00	ki19n	3.33±1.15
g1s	6.00±0.00	r19s	4.67±1.15	ku19s	2.67±1.15	kw1n	1.56±0.02	ks19s	1.67±0.58	ki19s	1.67±0.58
g20n	2.00±0.00	r1n	3.33±1.15	ku1n	0.00±0.00	kw1s	6.79±0.01	ks1n	4.67±1.15	ki1n	4.67±1.15
g20s	2.00±0.00	r1s	3.33±1.15	ku1s	5.33±1.15	kw20n	2.40±0.00	ks1s	5.33±1.15	ki1s	4.67±1.15
g21n	2.00±0.00	r20n	0.33±0.58	ku20n	0.33±0.58	kw20s	1.11±0.01	ks20n	0.67±0.58	ki20n	2.00±0.00
g21s	4.00±2.00	r20s	3.33±1.15	ku20s	2.00±0.00	kw21s	3.52±0.01	ks20s	1.00±0.00	ki20s	2.67±1.15
				ku21n	0.67±0.58	kw2n	2.41±0.01	ks21n	0.00±0.00		
				ku21s	4.00±0.00	kw2s	7.19±0.02	ks22n	1.00±0.00		

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Githurai		Ruiru		Kenyatta University		Kahawa Wendani		Kahawa Sukari		Kiwanja	
Sample	CC SCN (mg/L)±SD	Sample	CC SCN (mg/L)±SD	Sample	CC SCN (mg/L)±SD	Sample	CC SCN (mg/L)±SD	Sample	CC SCN (mg/L)±SD	Sample	CC SCN (mg/L)±SD
g22n	2.67±1.15	r21n	2.00±0.00	ku22s	1.00±0.00	kw3n	2.00±0.00	ks2n	2.35±0.02	ki21n	2.00±0.00
g22s	1.33±0.58	r21s	4.67±1.15	ku2n	0.00±0.00	kw3s	4.67±1.15	ks2s	2.35±0.01	ki21s	3.33±1.15
g23s	3.33±1.15	r2n	0.67±0.58	ku2s	2.67±1.15	kw4n	4.00±0.00	ks3n	2.93±0.05	ki2n	4.00±0.00
g2n	0.67±0.58	r2s	1.00±0.00	ku3n	6.00±0.00	kw4s	7.33±2.31	ks3s	4.45±0.00	ki2s	4.00±2.00
g2s	6.00±0.00	r3n	2.67±1.15	ku3s	6.00±0.00	kw5n	2.00±0.00	ks4n	1.88±0.01	ki3n	0.00±0.00
g3n	1.33±0.58	r3s	10.00±0.00	ku4n	2.00±0.00	kw5s	2.67±1.15	ks4s	3.59±0.00	ki3s	2.67±1.15
g3s	5.33±1.15	r4n	1.00±0.00	ku4s	5.33±1.15	kw6n	3.33±1.15	ks5s	4.69±0.01	ki4n	0.00±0.00
g4n	2.00±0.00	r4s	10.00±0.00	ku5n	3.33±1.15	kw6s	4.00±0.00	ks6n	2.75±0.02	ki4s	1.67±0.58
g4s	5.33±1.15	r5n	0.67±0.58	ku5s	4.67±1.15	kw7n	1.00±0.00	ks6s	1.24±0.01	ki5n	2.67±1.15
g5n	0.67±0.58	r5s	8.67±2.31	ku6n	3.33±1.15	kw7s	2.67±1.15	ks7n	1.59±0.03	ki5s	4.67±1.15
g5s	4.67±1.15	r6n	3.33±1.15	ku6s	2.67±1.15	kw8n	0.67±0.58	ks7s	1.96±0.01	ki6n	1.67±0.58
g6n	2.67±1.15	r6s	8.67±2.31	ku7n	0.67±0.58	kw8s	2.67±1.15	ks8n	1.45±0.05	ki6s	3.33±1.15
g6s	4.00±0.00	r7n	0.67±0.58	ku7s	5.33±1.15	kw9n	1.33±0.58	ks8s	2.35±0.00	ki7n	1.33±0.58
g7n	0.33±0.58	r7s	10.00±0.00	ku8n	2.67±1.15	kw9s	5.33±1.15	ks9n	1.18±0.01	ki7s	4.00±0.00
g7s	2.67±1.15	r8n	0.33±0.58	ku8s	3.33±1.15			ks9s	5.38±0.00	ki8n	2.00±0.00
g8n	1.00±0.00	r8s	2.67±1.15	ku9n	1.33±0.58					ki8s	4.00±0.00
g8s	3.33±1.15	r9n	1.33±0.58	ku9s	4.67±1.15					ki9n	0.00±0.00
g9n	1.33±0.58	r9s	2.67±1.15							ki9s	1.33±0.58
g9s	4.00±000										

CC = Colour chart

Table A2: Results of Lundquist method for smokers and non-smokers

	LUNDQUIST METHOD		LUNDQUIST METHOD
Sample no.	Smoker	Sample no.	Non-smoker
KS15s	2.50±0.01	R1n	3.67±0.00
R3s	9.99±0.02	R6n	3.04±0.00
KU20s	2.32±0.00	KU15n	7.40±0.01
R5s	8.51±0.02	R12n	3.67±0.00
KI16s	4.02±0.03	G11n	2.42±0.01
R3s	9.99±0.04	G21n	2.13±0.01
R7s	8.21±0.02	R7n	0.55±0.01
R4s	13.93±0.00	KS9n	1.17±0.00
R12s	7.15±0.00	KS11n	4.14±0.00
R18s	8.13±0.05	KS7n	1.59±0.00
KU4s	5.52±0.00	KW13n	2.97±0.00
G10s	5.38±0.00	KU3n	6.60±0.00
G11s	6.32±0.00	KW7n	1.02±0.00

APPENDIX II

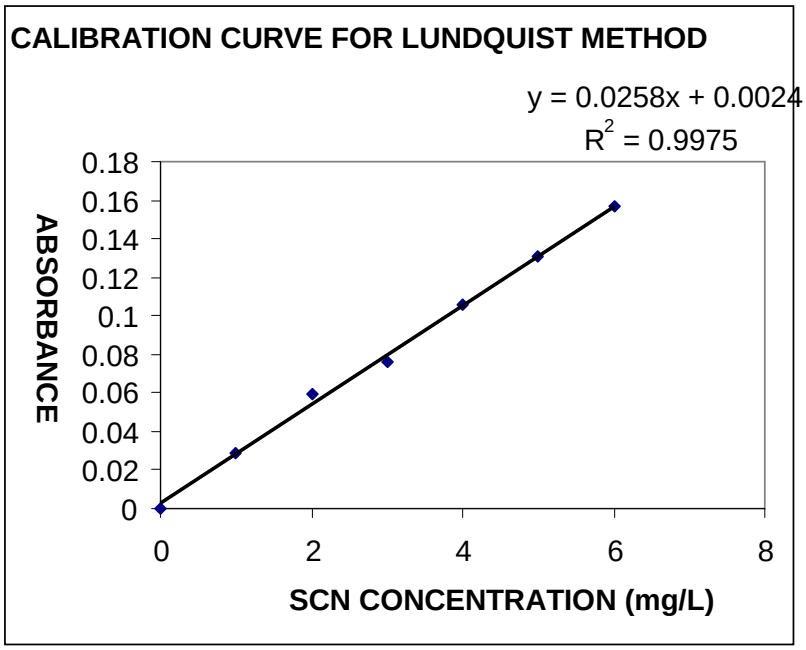


Figure A.1 SCN UV-visible calibration curve for Lundquist method

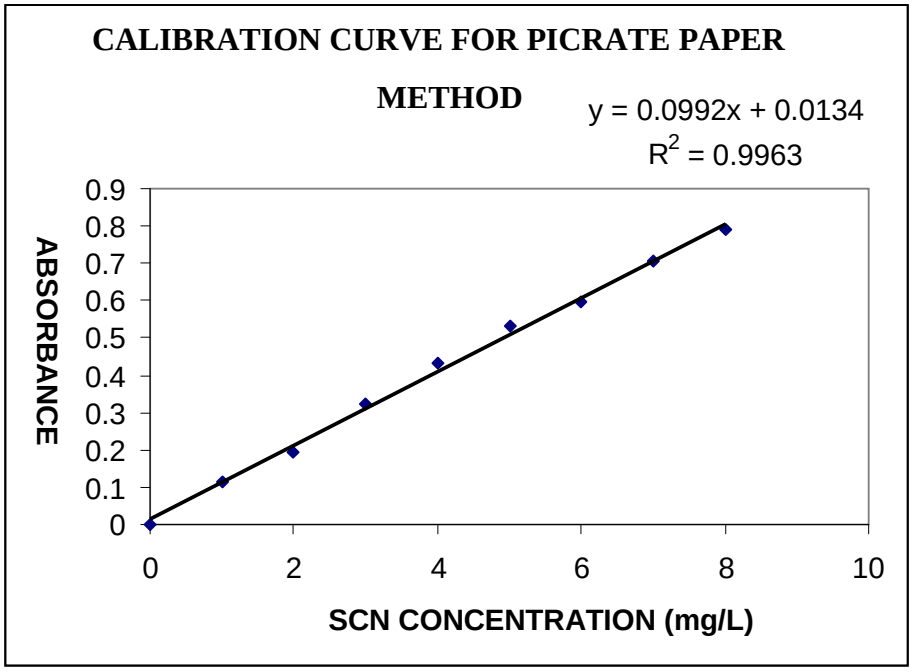


Figure A.2 SCN UV-visible calibration curve for picrate paper method

APPENDIX III

THE QUESTIONNAIRE

Date…….. No…….

This questionnaire is aimed at eliciting information, which will facilitate completion and writing of thesis report as part of a Master of Science degree program, department of chemistry in the School of Pure and Applied Sciences at Kenyatta University. It will also assist establish dietary habits and other factors which may influence the amount of cyanide in the bodies of people living in the study area. The information obtained will be strictly confidential and the name of the respondent will not be disclosed in whatever circumstance. Please give appropriate answers freely as sincere as possible. Thank you.

Instructions:
Please put a tick (√) in the box or fill in the blanks where applicable:

I) Personal information

1. Respondent's age, (years) ☐ <25 ☐ 25-45 ☐ 45-65 ☐ > 65

2. Gender ☐ Male ☐ Female

II) Environmental exposure

1. Where do you live?
   - ☐ Ruiru
   - ☐ Githurai
   - ☐ Kahawa – Sukari
   - ☐ Kahawa Wendani
   - ☐ Kenyatta University
   - ☐ Kiwanja

2. How long have lived in this area? (Years)
   ☐ 1-4 ☐ 4-8 ☐ >10

3. How do you dispose synthetic polymers (polythene bags, broken plastics)?
   ☐ Burn ☐ collect for recycle ☐ collect to municipal waste

III Cigarette smoke

1. i) Have you ever smoked cigarette?
   ☐ Yes ☐ No

a) If yes, are you still smoking or you stopped?
   ☐ Still smoking
   ☐ Stopped

b). If still smoking how many cigarettes do you smoke per day?
   ☐ 1 ☐ 2-5 ☐ 6-10 ☐ >1

c). How long have you smoked? (Years) ☐ <5 ☐ 5-20 ☐ >20

ii). If No in 1.i) above, have you ever been exposed to cigarette smoke from a member of your house you are living in or a person in the vicinity? ☐ Yes ☐ No

IV Dietary information

1. Which of the following foods make part of your diet more often?
   ☐ Cassava ☐ sorghum ☐ maize

2. For how long have you used the foods above? (Years)

   ☐ <5 ☐ 5-20 ☐ >20

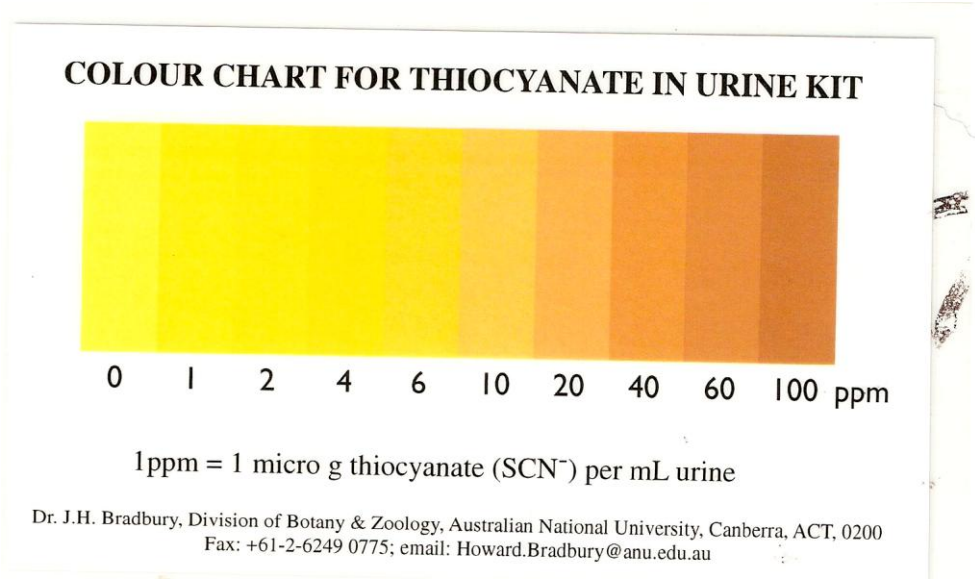
3. i) Incase of cassava or sorghum , how do you process them?
   ☐ Dry them and mill to flour
   ☐ Remove the outer peels of cassava and boil
   ☐ Chew them raw

   ii) Please specify any other way of processing these foods
   a) _____
   b) _____
   c) _____

Thank you, for your cooperation

APPENDIX IV

COLOUR CHART



APPENDIX V

RESEARCH PERMIT

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THIS IS TO CERTIFY THAT:

Prof./Dr./Mr./Mrs./Miss. NYACHOTI SYPROSE KEMUNTO

of (Address) KENYATTA UNIVERSITY P.O. BOX 43844 NAIROBI.

has been permitted to conduct research in

.....Location,

NAIROBI .....District,

NAIROBI .....Province,

on the topic DETERMINATION OF CYANIDE LEVELS IN URINE SAMPLES OF SMOKERS AND NON-SMOKERS IN SELECTED PARTS OF NAIROBI.

for a period ending 30TH AUGUST, 2008

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Research Permit No. MoST/13/001/37C765

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Applicant's Signature

Permanent Secretary Ministry of Science and Technology