KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

HEAVY METAL HEALTH RISK ASSESSMENT AND MICROBIAL QUALITY OF LOCALLY MILLED READY-TO-EAT TOMATO IN TAMALE, GHANA

BY:

ERNEST BONAH

NOVEMBER, 2014

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A THESIS SUBMITTED TO THE INSTITUTE OF DISTANCE LEARNING, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE (M.Sc. FOOD QUALITY MANAGEMENT)

BY

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NOVEMBER,2014 2014

DECLARATION

I hereby declare that the submission of this compilation is the true findings of my own researched work presented towards an award of an MSc. in Food Quality Management and that, to the best of my knowledge, it contains no material previously published by another person nor submitted to any other university or institution for the award of degree except where due acknowledgement has been made in text. However, references from the work of others have been clearly stated.

Ernest Bonah (PG8140112) **……..……** **……**

Student Name & ID **Signature** **Date**

Certified by:

Dr. Peter Twumasi **……** **…….**

Supervisor's Name **Signature** **Date**

Certified by:

Prof. (MRS) I. Oduro **……** **……**

Head of Depatment **Signature** **Date**

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ABSTRACT

This study was carried out with the aim to assess the microbial quality and risks associated with the intake of four heavy metals i.e., *lead (Pb), cadmium (Cd), arsenic (As) and mercury (Hg)* via the consumption of ready-to-eat tomato sauce from four (4) milling sites in the Tamale Metropolis . Twelve (12) samples of ready-to-eat tomatoes sauce were collected in triplicates from four (4) milling sites. The samples were digested with nitric and perchloric acid mixture (3:1) and analyzed using Atomic Absorption Spectrometer (AAS). Average concentrations (± SEM) of Cd, Pb, As and Hg in ready-to-eat tomato were found to be 0.1062±0.03330, 0.37085±0.19758, 0.00025±0.00004 and 0.00367±0.00068 mg/kg respectively. The concentrations of Cd and Pb were found to be significantly higher than the tolerable limits. However, concentration of Hg and As were within the permissible limits and thus safe to consume. Generally the level of health risk associated with exposure to the four heavy metals (Cd, Pb, As and Hg) through ingestion of the ready-to-eat tomato was low and does not increase for example cancer risk over a lifetime (70years). However, the mean dietary intake for Cadmium (15.61 µg/kg) and Mercury (47.57 µg/kg) were higher than the Provisional Tolerable Weekly Intake (PTWI) body of 2.5 and 25 µg/kg respectively. Analysis of the food samples also revealed mean total bacterial count to be 7.00 ± 0.46 $\log_{10}$ cfu/g. The minimum and maximum bacterial count ranged from 6.7 ±0.12 $\log_{10}$cfu/g to 7.2±0.9 $\log_{10}$ cfu/g respectively. The bacterial species encountered included *Bacillus sp*. and *Staphylococcus aureus*. Overall 66% of the samples tested Positive for *Escherichia coli*, this could be due contaminated processing water, or cross contamination of raw materials and cooked food by equipment or vendors. All the samples had bacterial counts higher than the acceptable levels and thus pose health risk to the local population who regularly patronize them.

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CHAPTER ONE

INTRODUCTION

1.1 Background to the problem

Sefa-Dedeh (2009) reported an increasing phenomenon of size-reduction services in the Ghanaian markets. These services are provided by people who use locally-manufactured equipment. The equipment are used for a multiplicity of food materials and are generally not washed between use and are potential sources of contamination of foods, especially freshly-ground mixture of pepper, tomato and onions used by *kenkey* eaters. The operators of these equipment whilst offering essential services do not follow any set of Sanitation Standard Operating Procedures (SSOP) to ensure the safety and quality of the foods they handle. The increasing interest in working in convenience at the expense of safety need to be addressed.

According to the FAO/WHO, (2007) street food is food obtained from a street side vendor, often from a makeshift or a portable stall. Street food plays an important role in the major urban cities in developing countries including Ghana. According to Tambekar *et al* (2008) street foods account for the feeding of thousands of city dwellers and it's relatively cheap and easily accessible.

Governments worldwide have initiated numerous attempts to improve food safety but food borne illnesses still possess a significant threat to health of people living in both developed and developing countries (WHO,2011).

In Ghana popular street foods include *ready-to-eat red tomator* (consisting of pepper, onions and tomatoes) normally eaten with kenkey, Fufu and Banku. Numerous literature suggest that food borne illnesses of microbial and heavy metal origins such as diarhoea have been recognised as the major source of hospital attendance in Ghana whilst in Africa, 16% of deaths among children under five years is directly attributable to diarrhoeal diseases (Bruce *et al.*,2005).

Street foods are major sources of enteropathogens (Mensah *et al.*, 2002). Feacal coliforms, including *Escherichia coli, Salmonella species, Bacillus Cereus, and Staphylococcus* have been recovered from water, hands and food contact surfaces in Ghana (FAO/WHO 2005).

Pathogenic microorganisms present in *ready-to-eat* foods indicates a need for quality assurance improvements by local producers in order to reduce consumer risk of exposure to infectious foodborne agents (Gibbons *et al.*, 2006). According to Gibbons *et al* (2006) ensuring good quality raw materials, adequate lethality treatment, and effective sanitation of both the equipment and processing environment are crucial in preventing all forms of food contamination.

Heavy metals are those elements which have density more than 5 g/cm^3, atomic weight 63.546 to 200.590 (Kennish, 1992) and a specific gravity greater than 4.0 (Connell and Miller, 1984).The uptake of dietary heavy metal among populations causes serious health problems including reduced immunological defenses, intrauterine growth retardation, impaired psycho- social behaviors, and disabilities associated with malnutrition and a high prevalence of upper gastrointestinal cancer (Arora *et al.*, 2008).

1.2 Statement of the problem

Ready-to-eat foods are considered high risk foods because they do not require heating before consumption. Inadequate sanitation in the processing areas might create favorable conditions for existing bacteria to flourish especially when good hygienic practices are not observed. Locally produced grinding machinery may also leach metals due to wear and tear.

Although governments throughout the world are attempting to improve the safety of the food supply, the occurrence of food borne diseases remains a significant health issue in both developed and developing countries (FAO/WHO, 2011). According to the (WHO 2011), contamination of food and drinking water contributed immensely to 1.8 million deaths from diarrheal diseases alone in 2005.

The apparent lack of information on the incidence and prevalence of diseases associated to street vended foods in developing countries does not provide sufficient data on the impact of street vended foods on human health (WHO, 2011). Most food vendors prepare or mill their food products in unhygienic and insanitary conditions due to lack of adequate knowledge on Good Hygienic Practices (GHP) (Sheth *et al*., 2005).According to Barro *et al* (2006) most consumers are more interested in convenience and thus pay little attention to hygiene, safety and quality of street vended foods. Feglo and Sakyi (2012) reported varying levels of *Staphylococcus aureus, Bacillus species, Klebsiella pneumoniae, Escherichia coli* in street food in the Kumasi metropolis. However the study did not focus on specific hazards posed by microorganisms of public health concern in street food.

Heavy metals however are one of the environmental pollutants of major concern as a result of industrial and commercial processes which have actively mined, refined, manufactured, burnt and manipulated heavy metal compounds for number of reasons. They are present in virtually every area of modern consumerism, from construction materials to cosmetics, medicine to processed food, fuel sources to agents of destruction appliances to personal care products.

Heavy metals are persistent in the environment and are subject to bioaccumulation in food chains. However exposure does not result only from the presence of a harmful agent in the environment. The key word in the definition of exposure is contact. Exposure is often defined as "an event that occurs when there is contact at a boundary between a human and the environment with a contaminant of a specific concentration for an interval of time (Berglund, 2001).

The purpose of this research therefore is to ascertain the contamination levels of microorganisms and the heavy metal index in foods processed by local mills in the Tamale metropolis.

1.3 Main Objective

The overall objective of this research was to determine the health risk associated with heavy metal consumption and the microbial quality of *ready-to-eat* locally milled tomato sauce.

1.4 Specific objectives:

1) To determine the presence and levels of lead, mercury, Arsenic, and Cadmium in ready-to-eat locally milled tomato sauce from the Tamale Metropolis.

2) To determine the presence and levels of food borne pathogens in ready-to-eat locally milled tomato sauce.

3) To measure the risk associated with exposure to heavy metals through ingestion of contaminated tomatoes.

CHAPTER TWO

LITERATURE REVIEW

2.1 Toxic effects of heavy metals

Heavy metals form a major global threat as they are present widely in the earth's crust, water, air and food (Matthew *et al*., 2002). According to Srikanth *et al* (2004), human activities such as mining, industralisation, and the use of agricultural pesticides coupled with natural activities has led to increased levels of heavy metal pollution in the ecosystem.

Human health risk associated with heavy metal intake has been widely studied due to the fact that it creates potential barriers for international trade. Guidelines and various regulatory frameworks for heavy metals in foodstuffs and the environment have been developed (McLaughlin *et al*., 2000). Whiles studies have shown that crops and vegetables grown in heavy metal contaminated soils have high heavy metal concentrations than those grown in uncontaminated soils. There has also been significant leaching of metals from machinery and equipment's used for processing crops and vegetables (Dowdy and Larson, 1995).Heavy metals exert either beneficial or harmful effects depending upon their chemical properties and concentrations.

Calderon (2000) noted that excess consumption of arsenic and cadmium which are non-essential trace elements resulted in several skin lesions, renal dysfunction, cardiovascular diseases and various cancers, even at very low doses. Another study in the Van region of Eastern Turkey by Turkgogan *et al* (2002), related the high prevalence of upper

gastrointestinal cancer in rats to high levels of Co, Cd, Mn, Ni and Cu present in fruits and vegetables in that region.

The presence of heavy metals in food and animal feed poses a severe risk and can lead to toxicological effects as a result of a long term exposure. Excessive exposure of elements such as cadmium, lead, arsenic, chromium and mercury is toxic for plants, animals and human beings (Llobet *et al*., 2003).

Tomlins *et al* (2004), reported high levels of cadmium, arsenic, mercury and copper in food samples suggesting possible leaching from the utensils as well as machinery. Local machinery for grinding vegetables and cereals are manufactured using scrap metal from diverse sources including industry machinery and vehicle parts. which are not food grade (Kwofie and Chandler, 2010).

2.1.1 Cadmium

Cadmium is a metal from group II B that has an atomic weight of 112.41with specific gravity of 8.65 (Singh, 2005). Cadmium is a soft, malleable, ductile, bluish-white bivalent metal and is highly carcinogenic for living beings. The Joint FAO/WHO has recommended the PTWI as 0.007 mg/kg/BW for cadmium (JEFCA, 2004). The EPA maximum contaminant level for cadmium in drinking water is 0.005 mg/L whereas the WHO adopted the provisional guideline of 0.003 mg/L (WHO, 2004a).

The Codex General Standard for contaminants and toxins in food and feed maximum levels of cadmium in fruiting vegetables is 0.05mg/kg. In general, for non-smokers and non-occupationally exposed workers, food products account for most of the human

exposure burden to cadmium (ExtoxNet, 2003). In food, only inorganic cadmium salts are present. Organic cadmium compounds are very unstable. In contrast to lead and mercury ions, cadmium ions are readily absorbed by plant and are equally distributed over the plant. Cadmium is taken up through the roots of plants to edible leaves, fruits and seeds. During the growth of grains such as wheat and rice, cadmium taken from the soil is concentrated in the core of the kernel. Cadmium also accumulates in animal milk and fatty tissues (Figueroa, 2008). Therefore, people are exposed to cadmium when consuming plant- and animal-based foods. Seafood, such as molluscs and crustaceans, can also be a source of cadmium (Castro-González and Méndez-Armenta, 2008).

Friberg *et al* (1979) and Zak and Steibert (1980), both reported in their respective studies that Cadmium compounds are extremely toxic for plants, animals and human beings having been found widely distributed in air, soil, water, plants and finally in animal tissues Bernard (2008), reported that cadmium did not have a single physiological function within the human body, thus diverting attention to the study of its bio hazardous potential. Once cadmium is absorbed it accumulates in the body throughout life. Nordberg *et al* (2007) also reported that low concentrations of cadmium can adversely affect the number of metabolic processes in animal body. Kidney, bone and pulmonary damages are attributable to cadmium intoxication (Godt *et al*., 2006). Cadmium is primarily toxic to the kidney, especially to the proximal tubular cells where it accumulates over time and may cause renal dysfunction. Cadmium can also cause bone demineralisation, either through direct bone damage or indirectly as a result of renal dysfunction. After prolonged and/or high exposure the tubular damage may progress to decreased glomerular filtration rate, and eventually to renal failure. (EFSA, 2009)

In animals, cadmium toxicity affects organs such as the liver, lung, testis and the hematopoietic system (Kocak and Akc, 2006). Literature suggests that high levels of cadmium in cattle leads to teratogenic effects, loss of appetite, anemia, poor growth, and abortions.

Powell *et al* (1964); Miller *et al* (1967); Neathery and Miller (1976); Doyle (1977); Wright *et al*., (1997); Bremner and Campbell (1978) have all reported alterations in the metabolism of copper and zinc in animals due to excessive intake of cadmium.

Wallace and Starkov (2000) and Sokolova (2004) reported that mitochondrial dysfunction is resulted due to cytotoxicity of cadmium. High cadmium doses are associated with not only human fatalities but gastric annoyance that results in emitting, abdominal pain and diarrhoea. Acute toxicity for cadmium in humans occur at 20 to 30 mg/kg which results in human fatalities.

Symptoms of acute toxicity include abdominal and muscular cramps, headache, overtiredness, shock and ultimately death (USAF, 1990).

Thompson and Bannigan (2008), also reported that cadmium exposure has deleterious effects on the kidney, vascular system and the liver; the study also recognised that reproductive tissues and developing embryos are the most undesirable effects of cadmium exposure on humans. The threshold limit for elemental cadmium, the oral LD50 values range from 63-88 mg/kg for cadmium chloride, 72 mg/kg for cadmium oxide and 590-1125 mg/kg for cadmium stearate (USAF, 1990).

Studies on cadmium toxicity in animals as well as in humans are well documented (Satoh *et al*., 2002; Thompson and Bannigan, 2008). Foodstuffs are the main source of cadmium exposure for the non-smoking general population. Cadmium absorption after dietary exposure in humans is relatively low (3–5 %) but cadmium is efficiently retained in the kidney and liver in the human body, with a very long biological half-life ranging from 10 to 30 years (EFSA, 2009).

The International Agency for Research on Cancer has classified cadmium as a human carcinogen (Group 1) on the basis of occupational studies. Newer data on human exposure to cadmium in the general population have been statistically associated with increased risk of cancer such as in the lung, endometrium, bladder, and breast.

Cadmium bioavailability, retention and consequently toxicity are affected by several factors such as nutritional status (low body iron stores) and multiple pregnancies, preexisting health conditions or diseases.

2.1.2 Mercury

Mercury is one of the most toxic heavy metals in the environment (Castro-González and Méndez Armenta, 2008). Mercury, in particular methyl mercury, poses a risk to public health, for example, it can affect the development of the brain of infants and can cause neurological changes in adults. (EFSA, 2004). Mercury is widely distributed within food as methyl mercury, its most toxic form, found at significant levels only in fish and seafood products.

Therefore, the levels of mercury and methyl mercury in food should be as low as reasonably achievable. A PTWI of 0.005 mg/kg (5µg/kg) bodyweight has been decided for total mercury. This is equal to 0.35 mg/week for a person weighing 70 kg.

Man released mercury into the environment by the actions of the agriculture industry (fungicides, seed preservatives), by pharmaceuticals, as pulp and paper preservatives, catalysts in organic syntheses, in thermometers and batteries, in amalgams and in chlorine and caustic soda production (Oehlenschläger, 2002; Zhang and Wong, 2007).

Exposure to high levels of metallic, inorganic, or organic mercury can permanently damage the brain, kidneys, and developing fetus (ATSDR, 2003b).The toxicity of mercury depends on its chemical form (ionic < metallic <organic) (Clarkson,2006). Up to 90% of most organic mercury compounds are absorbed from food (Reilly, 2007). Mercury can be detected in most foods and beverages, at levels of < 1 to 50 µg/kg (Reilly, 2007). Higher levels are often found in marine foods. Organic mercury compounds easily pass across biomembranes and are lipophilic. Therefore elevated mercury concentrations are mainly found in liver of lean species and in fatty fish species. Methyl mercury has a tendency to accumulate with fish age and with increasing trophic level. This leads to higher mercury concentrations in old fatty predatory species like tuna, halibut, redfish, shark, and swordfish (Oehlenschläger, 2002). In the year 2003, the JEFCA revised its risk assessment on methylmercury in fish and adopted a lower PTWI of 1.6µ g/kg body weight/week to replace the previous PTWI of 3.3 µg/kg b.w./week of total mercury for the general population (Castro González and Méndez-Armenta, 2008). This risk assessment was based on two major epidemiology studies which investigated the relationship between maternal exposure to mercury through high consumption of

contaminated fish and seafood and impaired neurodevelopment in their children (Grandjean *et al*., 1997; Murata *et al*., 2007). Because of the extreme health effects associated with mercury exposure, the current standards for drinking water were set by EPA and WHO at the very low levels of 0.002 mg/L and 0.001 mg/L, respectively (WHO, 2004a).

According to Montesinos *et al* (1997) mercury is capable of crossing the placental barrier and also secreted in milk. Mercury has no known vital or beneficial effect on the living organisms (WHO, 1976) and causes toxicity of the central nervous system in humans.

Ehmann *et al* (1986); Wenstrup *et al* (1990), showed through experiments performed on Alzheimer's disease patients elevated levels of mercury in various parts of brain and subcellular fractions. Another study by Basun *et al* (1991); Hock *et al* (1998) confirmed high concentration of mercury was found in blood and cerebrospinal fluid of Alzheimer's disease patients.

Overview of the above literature indicated that mercury is not only carcinogenic but also causes severe diseases, which ultimately end in death of human beings or animals.

2.1.3 Lead

The current annual world production of lead is approximately 5.4 million tons and still continues to rise (Howard, 2002) resulting in an intensive pollution of the environment with this metal. Dunham, (1972) reported that lead has been mined for centuries. Lead as a toxicologically relevant element has been brought into the environment by man in extreme amounts, despite its low geochemical mobility and has been distributed

worldwide (Oehlenschläger, 2002). Lead amounts in Deep Ocean waters is about 0.01-0.02 μg/L, but in surface ocean waters is ca. 0.3 μg/L (Castro-González & Méndez-Armenta, 2008). Lead still has a number of important uses in the present day; from sheets for roofing to screens for X-rays and radioactive emissions. Like many other contaminants, lead is ubiquitous and can be found occurring as metallic lead, inorganic ions and salts (Harrison, 2001). Lead has no essential function in man.

Food is one of the major sources of lead exposure; the others are air (mainly lead dust originating from petrol) and drinking water. Plant food may be contaminated with lead through its uptake from ambient air and soil; animals may then ingest the lead contaminated vegetation. In humans, lead ingestion may arise from eating lead contaminated vegetation or animal foods. Another source of ingestion is through the use of lead-containing vessels or lead-based pottery glazes (Ming-Ho, 2005). In humans, about 20 to 50% of inhaled, and 5 to 15% of ingested inorganic lead is absorbed. In contrast, about 80% of inhaled organic lead is absorbed, and ingested organic Pb is absorbed readily. Once in the bloodstream, lead is primarily distributed among blood, soft tissue, and mineralizing tissue (Ming-Ho, 2005). The bones and teeth of adults contain more than 95% of the total body burden of lead. Children are particularly sensitive to this metal because of their more rapid growth rate and metabolism, with critical effects in the developing nervous system (ATSDR, 2007b).

The Joint FAO/ World Health Organization Expert Committee on Food Additives (JEFCA) established a provisional tolerable weekly intake (PTWI) for lead as 0.025 mg/kg/BW (JEFCA, 2004). The WHO provisional guideline of 0.01 mg/L has been adopted as the standard for drinking water (WHO, 2004a).

2.1.4 Arsenic

Arsenic is a metalloid and exists in both organic and inorganic forms and raises concerns on both environmental and human health standpoints (EFSA, 2004).Foods contaminated with arsenical pesticides or grown with arsenic-contaminated water or in arsenic-rich soil are major sources of arsenic in humans. Nriagu *et al* (1990), indicated that foods grown in arsenic affected areas arsenic is not found inside the food like cereals, pulses, fruits, but may be found in external layer of food or fruits due to spreneld of arsenicated water.

Arsenic is a protoplastic poison due to its effect on sulfhydryl group of cells interfering with cells enzymes, cell respiration and mitosis (Gordon *et al*, 1948). In humans, soluble inorganic arsenic is rapidly and nearly completely absorbed after ingestion. After being absorbed, arsenic is widely distributed to almost all organs and readily crosses the placental barrier (EFSA, 2004).Biotransformation of inorganic arsenic in mammals includes reduction of pentavalent arsenic to trivalent arsenic and methylation of trivalent arsenic (EFSA, 2004).

The main adverse effects reported to be associated with long term ingestion of inorganic arsenic in humans are skin lesions, cancer, developmental toxicity, neurotoxicity, cardiovascular diseases, abnormal glucose metabolism, and diabetes (Chakraborti *et al.*, 2004). Neurotoxicity is mainly reported with acute exposure from deliberate poisoning or suicide, or at high concentrations in drinking water (Sakurai *et al.*, 2004).

There is emerging evidence of negative impacts of arsenic on foetal and infant development, particularly reduced birth weight. There is a need for further evidence regarding the dose-response relationships and critical exposure times for these outcomes.

The Agency for Toxic Substances and Disease Registry (ATSDR) in 2003 noted that inorganic arsenic is carcinogenic and has been related to skin and lung disorders. Chronic arsenicosis as an alarming environmental health disaster. The growing awareness of arsenic-related health problems has led to a rethinking of the acceptable concentration in drinking water (Sawyer *et al*., 2003). Following a thorough review and in order to maximize health risk reduction, the USEPA in 2001 decided to reduce the drinking water maximum contaminant limit (MCL) to 0.010 mg/L, which is now the same as the WHO guidelines (USEPA, 2005a).The JEFCA established a PTWI for inorganic arsenic as 0.015 mg/kg body weight (FAO/WHO, 2005, JEFCA 2004). Organo-arsenic intakes of about 0.05 mg/kg body weight/day seemed not to be associated to hazardous effects (Uneyama *et al*., 2007).

2.2 Theory of risk assessment

Since about 1970 the field of risk assessment has received widespread attention within both the scientific and regulatory communities (Paustenbach, 2002). Humans are regularly exposed to varied forms of harmful agents through the foods we eat, liquids we drink and the surfaces we come into contact with. One major public health intervention strategy is to reduce to extent to which humans are exposed to these harmful agents which contribute to increased rates of diseases, deaths or disability (WHO, 2000).For most developing countries like Ghana pursuing the principles of sustainable development, the assessment and management of risks from chemicals and microbial exposure must be a major priority (WHO, 1999).

Risk assessment as an effective tool for risk management has been fundamental for human survival over the last century (Hrudey, 1998). Risk assessment is defined by United States Department of Energy (DOE) as a tool used by decision-makers to assess the potential adverse human health effects that may result from exposure to contaminants at a particular site (DOE-ORO, 1999).

Health assessments include a preliminary assessment of risk to human health, identification of potential exposure pathways, the characteristics of the affected community, the short and long-term health effects for each chemical or microbial contaminant, and analysis of morbidity and mortality data on diseases caused by exposure to the contaminant. Many organisms have adapted and evolved diverse mechanisms to regulate long-term exposures and accumulations of heavy metals since they occur naturally (Fairbrother *et al.*, 2007).

The most important source of heavy metal exposure to humans is through the "econosphere" which contains food and drinking water (Baker *et al*., 2003). Risk assessment is categorised in four steps: 1. Hazard identification, 2. Hazard characterisation, 3. Exposure assessment and 4. Risk characterisation.

2.2.1 Hazard identification

Hazard identification is classified as the first step in risk assessment. According to Lammerding *et al*, (2001) hazard identification is a qualitative process that serves to document the important information about the chemical, pathogen, food product and host interface.

Hazard identification is defined in the Codex "Procedural Manual, Fourteenth Edition" (CAC, 2004) as "The identification of biological, chemical and physical agents capable of causing adverse health effects and which may be present in a particular food or group of foods"

Published literature, foodborne disease reports and epidemiological studies are sources used to identify hazards. Hazard identification also involve other aspects such as the types of disease caused and identifying the at risk populations and the mode with which the chemical or pathogen affects the host.

2.2.2 Hazard characterisation

Hazard characterisation is defined in the Codex "Procedural Manual, Fourteenth Edition" (CAC, 2004) as "The qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with biological, chemical and physical agents which may be present in food. For chemical agents a dose–response assessment should be performed. For biological or physical agents a dose–response assessment should be performed if the data are obtainable.

2.2.3 Exposure assessment

Exposure assessment is defined in the Codex "Procedural Manual, Fourteenth Edition" (CAC, 2004) as "The qualitative and/or quantitative evaluation of the likely intake of biological, chemical and physical agents via food as well as exposures from other sources if relevant".

An exposure assessment estimates qualitatively or quantitatively the magnitude, frequency, duration and the route of exposure for each potential or actual population to be evaluated in the risk assessment (DOE-ORO 1999).

This step should include a site characterization, identification of the potential exposure pathways and quantification of actual or potential exposure (DOE-ORO 1999).

2.2.4 Risk characterisation

Risk characterisation is defined in the Codex "Procedural Manual, Fourteenth Edition" (CAC, 2004) as "The qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterisation and exposure assessment".

Risk characterisation brings together all of the qualitative or quantitative information of the previous steps to provide a soundly based estimate of risk for a given population. These steps involve the compilation of data from the previous steps and its incorporation into a mathematical model to derive a value for risk with these models changing significantly depending on several factors (LeCoultre, 2001).

According to LeCoultre (2001) these factors may include daily intake value, exposure level, RfD, RfC, specific data concerning the people exposed (e.g. age, body weight, inhalation rate, etc.), chemical specific constants such as uptake factors, absorption factors, and residency time. Assumptions and generalizations are used because it is impractical to determine the exact values for each site, each chemical, and each

potentially exposed individual. Uncertainty factors are incorporated into the model to account for these issues and the variability of the toxic effects of chemicals.

2.3 Microbial quality of street foods

Street foods are ready-to-eat foods and beverages prepared and/or sold by vendors and hawkers especially in streets and other similar public places, FAO (1989).

Street foods are highly patronized for their convenience, nutritional quality and flavors, they also play a huge role in providing income for non-skilled city dwellers contributing to the economies of developing countries.

Street foods are considered a public health risk due to the difficulty in controlling the large numbers of street food vending operations because of their diversity, mobility and temporary nature (DeSausa ,2008) and the lack of basic infrastructure and services (Ghosh *et al.,* 2007).

Mensah *et al*, 2002 reported that hygienic aspects of vending operations are a major source of concern for food control officers siting the unavailability of portable water, lack of toilet facilities, hand washing stations as examples.

Epidemiological studies conducted on street foods suggest that street food contributes significantly to diarrhoeal infection and food poisoning outbreaks due to poor hygiene practices, lack of knowledge about important parameters in the food chain and host pathogen interactions (Tambekar *et al*., 2008).

Microbial safety of street foods poses a threat to public health because vending is done in places that have poor sanitation (Barro *et al*., 2006).

Street foods in some African countries have been tested for various microorganisms of public health concern, including *feacal coliforms, Escherichia coli, Staphylococcus aureus, Salmonella spp* and *Bacillus cereus* and *S. aureus.*

Mensah *et al*., (2002) examined 511 street foods in Accra and reported the presence of mesophilic bacteria, *Bacillus cereus, E. coli, Staphylococcus aureus, Enterobacteriaceae* and *Shigella sonnei* in most street foods.

Umoh and Odoba, (1999) observed that over 26% of street food samples analyzed in Nigeria contained B. cereus, while 16% contained S. aureus. These observations indicate that although street foods are a major source of nutritious food, they are also a possible source of food poisoning microorganisms.

Bruce *et al* (2005), reported that diarrhoeal diseases are the major causes of hospital attendance in Ghana. Studies conducted in Kumasi have also identified vegetables prepared by street food vendors to be highly contaminated with faecal material and harmful micro-organisms (Amoah *et al*., 2006) and several related risk practices of food handling have been identified by Henseler (2005) and Olsen (2005).

2.3.1 Bacteria pathogens associated with food borne diseases

Foodborne pathogens are a major public health hazard of street foods due to their method of preparation and conservation (FAO/WHO 2005). Mensah *et al* (2002) and Barro *et al* (2006), reported that traditional methods of cooking, inadequate temperature holding time

and poor hygiene practices where the major sources of contamination in street vended foods. Recent studies conducted by Mankee *et al* (2005) and Christison *et al* (2008), indicated that food preparation surfaces and machinery are the major sources of microbial contaminants.

The epidemiological importance of microbial foodborne diseases of street foods cannot be underestimated due to our reliance on fast foods. All age groups consume street foods in Africa (FAO/WHO 2005). Children under five have experienced serious health implications due to the consumption of street foods bought by their mothers at markets in Accra (Mensah *et al*., 2002).

2.3.2 Coliforms

Coliforms represents a group of species from several bacteria including Escherichia, *Enterobacter, Klebsiella, citrobacter* and probably *Aeromonas* and *Serratia* (Feng *et al*.,2001). These groups of bacteria are all Gram negative non-spore forming rods; many are motile and are facultative anaerobes resistant to many surface active agents. These are able to grow in the presence of bile salts or other replacement surface active agents having an analogous growth inhibitory effect and that ferment lactose with gas and acid (or aldehyde) production within 48 h at 37 ± 1 C (Yousef and Calstrom, 2003).

Yousef and Calstrom, (2003) indicated that all these species are able to grow in foods except those that are at pH ≤ 4.0 and water activity ≤ 0.92, they are sensitive to low heat treatments and are killed by pasteurisation.

Studies conducted by Ray (2000) indicated that coliforms are present in some plants products in high numbers due to soil contamination reducing the specificity of coliforms as an indicator of fecal contamination for raw foods since coliforms in the food may result from growth of small non fecal coliforms.

Ray (2004) also indicated that in heat processed food products, the presence of coliforms did indicate post-heat treatment contamination from improper sanitation.

2.3.3 Fecal coliforms

Fecal coliform are a group of bacteria that includes coliforms whose specificity as fecal contaminants is much higher than coliforms. This includes *Klebsiella, Enterobacter* and *E coli*. Hoadley and Dutka, (1977) indicated that the improved specificity of *fecal coliforms* index as compared to coliforms lead to its widespread acceptance and use.

Ray (2004) did indicate that *fecal coliforms* are present in raw foods of animal and plant origin from contaminated soil or water. He reported that high numbers of fecal coliforms could be as a result of cross contamination, storage at abusive temperatures and growth form a low initial level.

The presence of fecal coliforms in *ready-to-eat* foods is attributable to improper sanitation and contaminated water and may suggest fecal contamination and the presence of enteric pathogens (Eijkman *et al* 1904).

The detection of *fecal coliforms* is done under high incubation temperature (44.5°C) for 24h in selective broths containing lactose.

2.3.4 Escherichia coli

Escherichia Coli is a member of the family *Enterobacteriiaceae* (Conway, 1995) consisting of many genera, the pathogens *Salmonella, Shigella* and Y*ersinia* all inclusive. According to Gassama *et al* (2001) most *E. coli* strains are not regarded as pathogens, however a few strains are opportunistic causing infections in immunosuppressed host.

Most pathogenic *E. coli* are grouped as either been enteropathogenic, enterohaemorrhagic, enteroaggregative, enterotoxigenic and enteroinvasive. The enteropathogenic strain (e.g. *E. coli 0157:H7*) causes gastrointestinal illness in health individuals (Ray, 2004).Shardinger in 1892 proposed the use of *E. coli* as indicator of fecal coliforms based on the abundance of *E.coli* in human and animal feces (BAM, 2002).

2.3.5 Staphylococcus aureus

Staphylococcal food intoxication is estimated to cause 185,000 cases of foodborne illness annually (Mead *et al*., 1999). Bergdoll, (1979) stated that unlike other foodborne illnesses, which usually have longer incubation periods, the onset of staphylococcal foodborne illness may occur between 30 min and 8 hr. following consumption of the toxin-containing food.

Studies conducted by Loir *et al* (2003) indicated that in all cases of Staphylococcal food poisoning of food, the enterotoxin producing S. aureus strain was exposed to temperatures suitable for the growth of *S. aureus* .Many different types of foods

implicated in Staphylococcal food poisoning of food include salads, vegetables, sandwich fillings and cooked meals (Olsen,2000).

According to Olsen (2000) the main source of contamination are improper handling of food by contaminated humans or through indiscriminate coughing or sneezing. *S. aureus* is found in approximately 30 to 50% of the human population (Di Giannatale *et al*., 2012). A study conducted in Austria revealed that 3.8% of the tested food contained coagulase positive *S. aureus* (EFSA, 2012). Kim *et al* (2011) reported high numbers of *S. aureus* in *ready-to-eat* Korean foods.

As a Gram positive, facultative anaerobe most of its vegetative cells are not heat stable but the formed toxins are not easily destroyed by heat causing food safety problems (Argundín *et al*., 2010). These enterotoxins that are produced by S. aureus in food are ingested by humans. According to Balaban and Rasooly, (2000) these toxins are produced in the mid exponential phase of the growth of *S. aureus*. Staphylococcal infections are dependent on the different virulence factors while with food poisoning staphylococcal enterotoxins is the main virulence factor (Le Loir *et al*., 2003). Apart from food poisoning, *S. aureus* can also cause infections such as pimples, furuncles, sepsis and toxic shock syndrome.

2.3.6 Bacillus Species

Bacillus is a Gram-positive, aerobe or facultative anaerobe bacterium that is widely spread in the environment. It has the ability to form spores therefore they can survive in a wide range of stress conditions (Senesi and Ghelardi., 2010).

Stenfors Arnesen *et al* (2008) in their study did indicate that *B. aureus* are easily transferred from the environment to food.

B. cereus causes a number of infections such as meningitis, brain abscesses, endophtalmitis, pneumonia, etc. (Bottone, 2010) and two different types of food poisoning. The bacterium has two types of toxins with different symptoms: the emetic toxin and the diarrheal toxin.

The emetic toxin (cereulide) causes vomiting and has been linked to lethal food poisonings (Mahler *et al*., 1997; Dierick *et al*., 2005) and the more prevalent diarrheal toxins (Arnesen *et al*., 2008).

Modern ready-to-eat meals especially vegetables undergo no heating to ensure the high quality of the product. Vegetative cells of *B. cereus* are largely eliminated by this mild heat processing, though the spores of some heat-stable *B. cereus* are able to survive (Samapundo *et al*., 2011). These spores are then able to germinate under favorable conditions and form enterotoxins in the small intestine of the host (Wijnands *et al*., 2002).

B. cereus is mostly found in decaying matter, vegetables and the intestinal tract of invertebrates. Ceuppens *et al* (2011) in his study observed that the enterotoxins preformed do not play a role in food poisoning because they are sensitive to heat and to gastrointestinal passage. The pH of the stomach influenced through the type of food consumed as well as the age of the individual can promote B. cereus enterotoxins. It is therefore important to pay more attention to the elderly who have a weakened immune system (Stenfors Arnesen *et al*, 2008). Elevated pH can help protect preformed toxins

and cause food poisoning. The spores of *B. cereus* are capable to survive extreme environmental conditions such as heat, freezing, drying and radiation (Bottone, 2010) and even the lower pH of the stomach, which makes the spores very persistent.

2.3.7 Ready-to-eat foods at retail level

Ready-to-eat foods refers to food that is in the form that is edible without cooking, washing or heating by the consumer and that is reasonably expected to be eaten in that form (USDA, 2002).

Vegetables such as *ready-to-eat* blended tomatoes are considered *ready-to-eat* foods. This category of foods are considered high risk since food workers, food contact surfaces etc can transmit pathogens onto the food. (Gombas *et al*, 2003).

Gombas *et al* (2003) conducted a survey of Listeria monocyotogenes in *ready-to-eat* foods from retail markets and indicated higher frequencies of Listeria monocyotogenes in in-store packaged meats than manufacturer packaged products in Maryland, USA.

Yapp and Fairman, (2006) reported that street vending in poverty stricken areas will have limited resources to train their staff on safe handling practices and guarantee the safest food supply to consumers.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

The study was conducted in the Northern regional capital of Ghana, Tamale. Tamale is situated at 9.4° North latitude, 0.83° West longitude. Tamale has a projected population of 537,986 according to the 2010 census. Four milling plants in four location in the metropolis namely Kukuo (MILL A), Choggu Hiiltop (MILL B), Lamshegu (MILL C) and the central bussiness district (MILL D). Common foods milled at these local mills include tomatoes, kenkey, ginger, pepper or a combination of some

. 3.2 Sample collection

A total of twelve (12) samples of ready-to-eat tomato from four (4) different milling sites sampled in triplicates over a three week period in the Tamale metropolis were used for the study.

3.3 Preparation and dry ash digestion of plant tissues for elemental analysis

The sample was weighed into a clean ceramic crucible. An empty crucible was included for a blank in each batch of 24 samples. The samples were arranged in a cool muffle furnace and temperature ramped to 300 degree Celsius over a period of 2 hours. This temperature was maintained constant for an additional 2 hours. The samples were allowed to cool down in the furnace.

Each sample was then removed from furnace ensuring that the environment is free from breeze. The ashed sample was transferred first into already numbered 50 ml centrifuge tubes. Crucibles were rinsed with 10 ml of distilled water into the centrifuge tubes. More rinsing of the crucible with 10 ml of aqua regia was done. Each sample was shaken for 5 minutes for proper mixing on a mechanical reciprocating shaker. Each sample was then centrifuged for 10 minutes at 3000 rpm and then decanted into 100 ml volumetric flasks and again made up to the 100 ml mark with distilled water. The clear supernatant digest were transferred into clean reagent bottles for arsenic, cadmium, mercury and lead determinations. A lower ashing temperature (300 degree Celsius) was used during digestion to avoid measurable volatisation of mercury and Arsenic.

3.3.1 Determination of Cadmium (Cd), Mercury (Hg), Arsenic (As) and lead (Pb) By Atomic Absorption Spectrometer (AAS)

The basic setup (air pressure = 50 – 60 psi, acetylene pressure = 10 -15 psi and voltage = 208 – 240V) of the AAS was ensured. The file for the type of analysis and hollow lamp was selected with appropriate wavelengths - Cd at 228.9 nm, and Pb at 283.3 nm. A calibration curve was plotted for each of the elements to be analyzed from the stock standards (Buck Scientific). The prepared sample solutions from 1.0 above were analyzed for the elements Cd and Pb. The Y in the calibration equation is absorbance of the element and X is the concentration of the element in the sample. X was calculated after substituting the absorbance reading of the sample into the calibration equation. This gave X in terms of mg/L. The total concentration of the element in the sample solution (100 ml) was calculated by multiplying the concentration in mg/L by 0.1 L. This gave the total mass of the element in solution. The percentage amount of the element was found by

dividing the mass of the element in solution by initial amount of sample taken followed by a multiplication by 100.

3.4 Microbial culture analysis

The samples were diluted by adding five grams of food to 50 ml of buffered peptone water and then shaken vigorously to dislodge adhered bacteria. The liquid phase then forms the stock sample from which dilutions were made to obtain 10^1, 10^2, 10^3 up to 10^{10} dilutions. After the dilution, 0.1 ml of it was transferred onto a sterile plate count agar (PCA) (Oxoid Ltd, Basingstoke Hants, England) then spread on the agar surface and immediately placed in an incubator. The plates were incubated at 37 ^{0}C over-night. The remaining stock samples were incubated at 37 ^{0}C for 4 hours after which they were subcultured onto MacConkey Agar (Oxoid Ltd, Basingstoke Hants, and England) plates and incubated at 37 ^{0}C overnight.

3.4.1 Viable bacterial count

After overnight incubation, growth on the PCA showing 30-300 colonies was counted. Bacterial counts were ex-pressed as the log of colony-forming-units per ml for liquid food or per g for solid food sample analyzed.

3.4.2 Bacterial identification and biochemical test.

The MacConkey and Nutrient agar plates were examined for bacterial growth. Growth characteristics and other colonial morphology such as lactose fermentation, formation of mucoid colonies of the bacteria were carefully recorded. Less than five identical colonies for a particular organism growing on a plate were ignored. When more than five similar

colonies were counted on a plate, then five isolated identical colonies on either the blood agar or MacConkey agar plates were picked carefully, one by one and inoculated into buffered peptone water in sterile microtitre wells. Culture from each microtitre well was re-inoculated onto a Nutrient agar (Oxoid Ltd, Basingstoke, Hamspshire, England) to obtain pure growth. Organisms which were identified to be the same from the microtitre wells were grouped as one isolate from the food sample analyzed. Bacterial identification was done using the pure culture on the nutrient agar plates. The first test was the Gram staining and the results were followed by the appropriate biochemical tests

For identifying *Escherichia Coli*, Eekman test was used. In this test, the sample was cultured in green broth and peptone water and after 24 hours of incubation, drop of the Kovacs reagent was added to tubes containing peptone water medium. A red color ring on the tube, indicate a positive test for Escherichia *coli.*

3.5 Determination of dietary intake

Three hundred (300) food frequency questionnaires (FFQ) based on tomato sauce consumption were used to estimate the daily and weekly consumption of the tomato thus determine their dietary intake and exposure to As, Cd, Pb and Hg from consumption of *ready-to-eat* locally milled tomato sauce. These FFO's were distributed to different categories of the populace comprising school children, pregnant women, lactating mothers, adult men and adult women purchasing *Kenkey* across the study sites. Additional information on socio-demographic data for each respondent was also given in the questionnaire. The body weight of the subjects were taken and recorded accordingly. Photographic method (a two way dimensional picture) was used in estimating the portion

sizes. The different portions of the puree served of were purchased, and classified into different sizes ranging from large, moderate and small. A digital camera was used to photograph the different sizes of Tomato sauce which was attached to the questionnaire. The tomato sauce were weighed and recorded accordingly.

3.6 Statistical analysis

One way analysis of variance (ANOVA) was used to determine any significant difference at 5% probability of the studied metals and microbes in the various samples while Monte-Carlo simulation was used to estimate the dietary intake of the heavy metals by the populace.

3.7 Risk assessment

The probability of getting cancer (not the probability of dying of cancer) and the associated dose, consist of an average taken over an assumed 70-year human lifetime. This dose is called the lifetime average daily dose or chronic daily intake.

To accommodate the uncertainties associated within the calculation process, the health risks for the local population due to exposure to metals were evaluated using Monte Carlo simulation technique was used based on @RISK software (Palisade, US) and considering 10,000 iterations. Before this process, distribution characteristics of each exposure parameter were tested according to the exposure results. A probabilistic distribution of the exposure dose was then obtained as simulation result.

The use of Monte Carlo simulations in risk assessment provides an understanding of the degree of uncertainty and variability around a risk estimate that single-point estimates of risk cannot provide (EPA 1994b).

The following Assumption was made for the

ED = 70-year lifetime for carcinogenic effects (*i.e.*, 70 years × 365 days/year)

Exposure duration was throughout the year since consumption is taken place every day of the year.

The mean exposure concentration of contaminants was used with exposed population variables and the assessment determined variables to estimate contaminant intake. The general equation for chemical intake is:

$$CDI = \frac{C \times CR \times EFD}{BW} \times \frac{1}{AT} \qquad \text{equation 1}$$

Where:

CDI = Chronic daily intake; the amount of chemical at the exchange boundary (mg/kg-day)

C = average exposure concentration over the period

CR = contact rate, the amount of contaminated medium contacted per unit time

EFD = exposure frequency and duration, a variable that describes how long and how often exposure occurs. The EFD is usually divided into two terms:

EF = exposure frequency (days/year) and

ED = exposure duration (years)

BW = average body mass over the exposure period (kg)

AT = averaging time; the period over which the exposure is averaged (days)

The chronic daily intake is simply compared with the RfD, then, if the CDI is below the RfD, it is assumed that the risk is negligible for almost all members of an exposed population.

Risk of chemical toxin is calculated using equation 2

$$Risk = PF(CDI - RfD)$$

Where

$PF\ is\ potency\ factor\ of\ the\ toxin\ and\ RfD\ is\ the\ reference\ dose\ of\ the\ toxins$

However in the absence of the reference dose of toxins (thus if a little of such reference toxins availability is assumed to have a risk, then there will be no level of which it is assume as safe for consumption, therefore RfD = 0)

$$Risk = PF * CDI$$

CHAPTER FOUR

RESULTS

4.1 Mean concentrations of heavy metals in the tomato samples

The mean concentrations for each metal, over the entire span of the sampling sites are shown in the table below.

Table 1: Mean concentration and standard error of mean of each metal analysed (mg kg-1).

Sampling site	Arsenic	Cadmium	Mercury	Lead
MILL A	0.0003±0.00006	0.1558±0.13427	0.0047±0.00318	0.1796±0.06997
MILL B	0.0002±0.00000	0.0926±0.159	0.0032±0.004	0.2227±0.056
MILL C	0.0003±0.00000	0.0926±0.159	0.0036±0.00289	0.5180±0.055
MILL D	0.0002±0.00006	0.0839±0.13587	0.0036±0.00289	0.5630±0.27556
Mean	**0.0003±0.00004**	**0.11±0.03330**	**0.004±0.00068**	**0.4±0.19758**
FAO/WHO STANDARD	**0.1**	**0.05**	**0.03**	**0.2**

The metal levels in all study areas showed the order Pb >Hg>Cd>As (Table 1).

The mean concentration of each element is shown in figure 1. The analytical results showed that all the studied elements were detectable. Bars with different letters show statistical differences. (n=12; bars represent standard error of means).

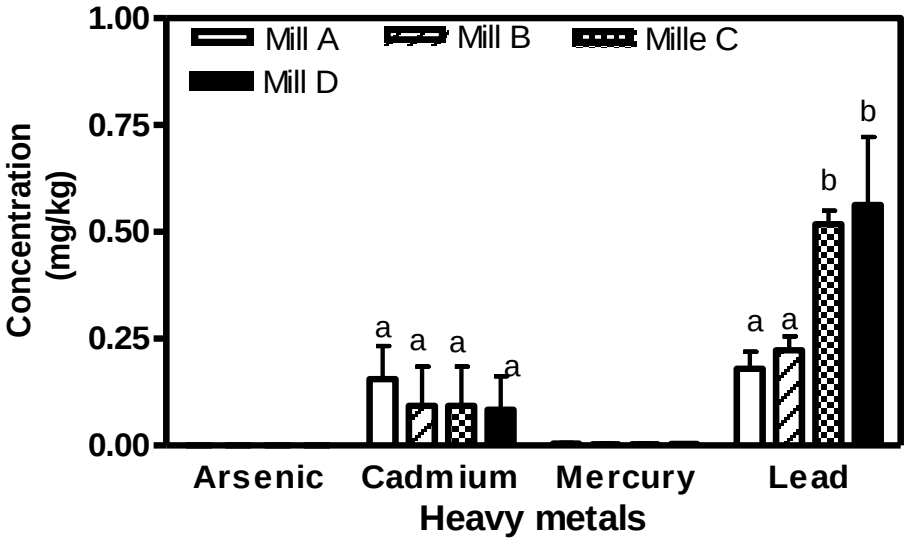

Figure 1: Concentration of heavy metals in tomato sauce collected from four different Tomato Mill in the Tamale Metropolis.

MILL A contained the highest concentration of the metals As (0.0003mg/kg), Cd (0.1558 mg/kg), and Hg (0.0047mg/kg) but recorded the least concentration for Pb (0.1796 mg/kg) while the central market had the least concentration except for Pb (0.5630 mg/kg).

The concentration of Pb in this study exceeded the FAO/WHO standard of 0.2mg/kg. The concentration of Cd (0.1062 mg/kg) was slightly above the WHO reference standard of 0.050 mg/kg.

Arsenic mean concentration in ready-to-eat tomatoes was found to be far below the WHO/FAO (2007) standard of (0.1mg/kg) (Table 1). The minimum and maximum concentration measured was (0.0002±0.000 mg/kg) and (0.0003±0.00006 mg/kg) respectively.

Cadmium has the lowest maximum levels (MLs) in foodstuffs for vegetables 0.050 mg/kg. All the samples collected from the milling sites was above the maximum levels. The minimum and maximum values been 0.0839 mg/kg and 0.1558 mg/kg respectively.

The mean concentration of mercury in this study had a minimum concentration of (0.0032±0.0004 mg/kg) and a maximum of (0.0047±0.00318 mg/kg) and an average of (0.00367±0.19758) for all samples. These values are all below the permissible limits of 0.03mg/kg.

The minimum and maximum mean concentration of Lead in this study was (0.1796±0.066997 mg/kg) and (0.5630±0.27556 mg/kg) respectively. The average mean concentration for lead of the sample sites was (0.37085±0.19758 mg/kg).

4.2 Risk assessment

4.2.1 Estimation of dietary intake/exposure

The data from the questionnaire was categorized and converted to show the mean daily consumption of the *ready-to-eat* tomato sauce in gram per person per day (Figure 2), the 95^{th} percentile of respondents as shown in Figure 3 consumed was 0.02541 kg of ready-to-eat tomato or less and 5% consumed 0.01691 kg or more. The average mean consumption was 0.02100 kg with a standard deviation of 2.3. Maximum and minimum consumption per day was 0.015 and 0.030 kg respectively.

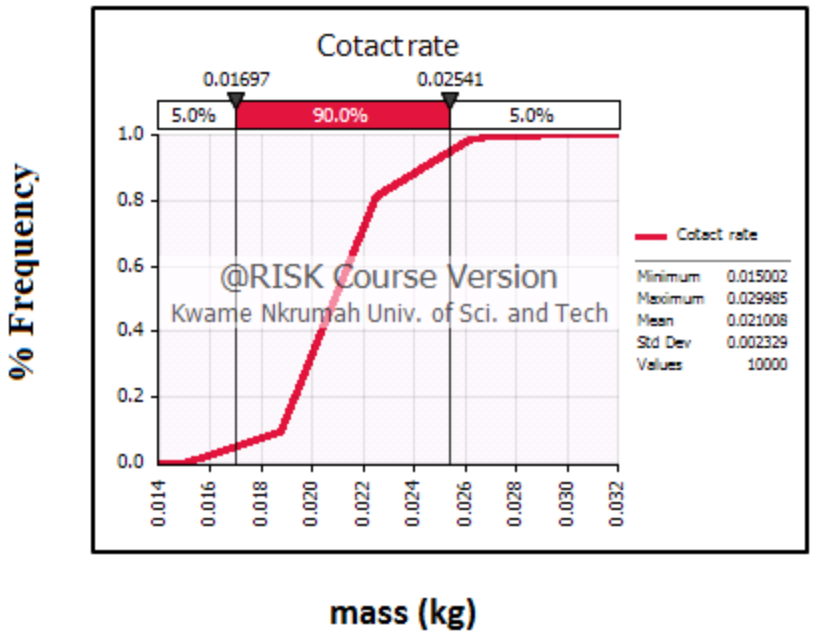

Figure 2: Riskhistogram distribution of mean daily consumption (kg/person/day)

The weights of three hundred respondents were measured in the survey. Forty –two percent (42%) of respondents had weights above 70 kg and 24.6 % had weights between 61 kg and 70kg. The remaining had percentages of 2.7%, 7.43%, 22.64% , 24.66% and 0.68% respectively for weights between 40 – 45,46 -52,53-60 and below 40 kg.

Table 2: Weight distribution

WEIGHT	Frequency	Percent	Cum. Percent	95% CI Lower	95% CI Upper
< 40	2	0.68%	0.68%	0.08%	2.42%
>70	124	41.89%	42.57%	36.21%	47.74%
40-45	8	2.70%	45.27%	1.17%	5.26%
46-52	22	7.43%	52.70%	4.72%	11.04%
53-60	67	22.64%	75.34%	17.99%	27.83%
61-70	73	24.66%	100.00%	19.86%	29.98%
TOTAL	**296**	**100.00%**	**100.00%**		

The mean body weight (kg) (Figure 3) was was 69.6 kg. From the distribution, the 95th percentile of the weight was 79.20 kg. This means that 95% of the adult population of the Ghanaian subpopulation in Tamale weighs 79.20 kg or less. The remaining 5% had weights of 54.78 kg or more.

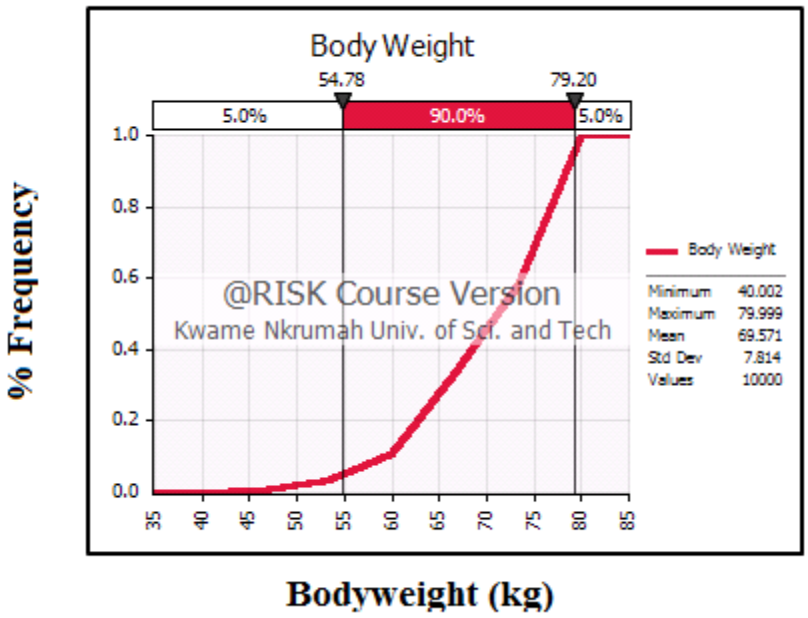

Figure 3: RiskHistogram distribution of Mean Bodyweight.

4.3 Mean dietary exposure

Risk to health in this study was assessed using Montecarlo simulation and comparing estimates of dietary exposure with the Provisional Tolerable Weekly Intakes (PTWIs) recommended by the Joint FAO/WHO Expert Committee on Food Additives (JEFCA) as shown on Table 3.

Table 3: Mean dietary intake of heavy metals and their Provisional Tolerable Weekly Intake.

Heavy metal	Mean metal intake µg/kg	Mean daily consumption g/person/day	Mean weekly consumption g/person/wk	Mean dietary µg/p/wk	Mean dietary µg/kg body wt/wk	Provisional Tolerable Weekly Intake (PTWI) µg/kg body weight
Arsenic	0.0003	21.0	147	0.04	0.0006	15
Cadmium	0.11	21.0	147	16.2	0.2	2.5
Mercury	0.004	21.0	147	0.6	0.01	4
Lead	0.4	21.0	147	58.8	0.8	25

4.4 Distribution of heavy metals in ready-to-eat tomato sauce.

4.4.1Arsenic

The daily amount was found to be vary with minimum of 0.0002mg/kg and the maximum of 0.0003mg/kg. The average concentration of arsenic was found to be 0.00025mg/kg.

A uniform distribution was set on the amount of Arsenic, using the minimum arsenic content and the maximum arsenic content since the selection of tomato sauce to a consumer is random, hence any of the tomato sauce with an unknown specific amount will be given to a consumer.

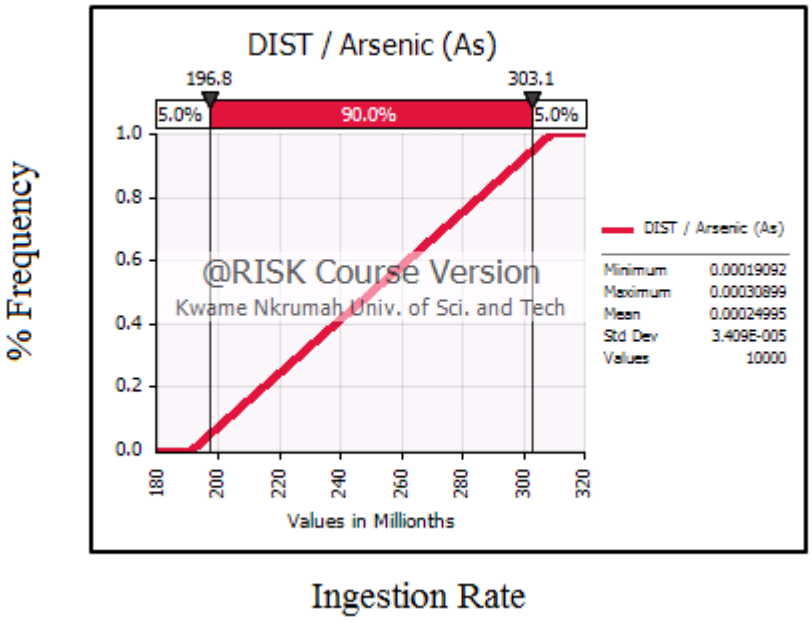

Figure 4: RiskUniform distribution on mean Arsenic intake (mg/kg).

4.4.2 Cadmium

The daily amount was found to vary with minimum of 0.0001mg/kg and the maximum of +∞. The average concentration of cadmium was found to be 0.00057mg/kg.

A Pareto distribution was set on the amount of Cadmium, using the minimum cadmium content and the maximum arsenic content since the selection of tomato sauce to a consumer is random, hence any of the tomato sauce with an unknown specific amount will be given to a consumer.

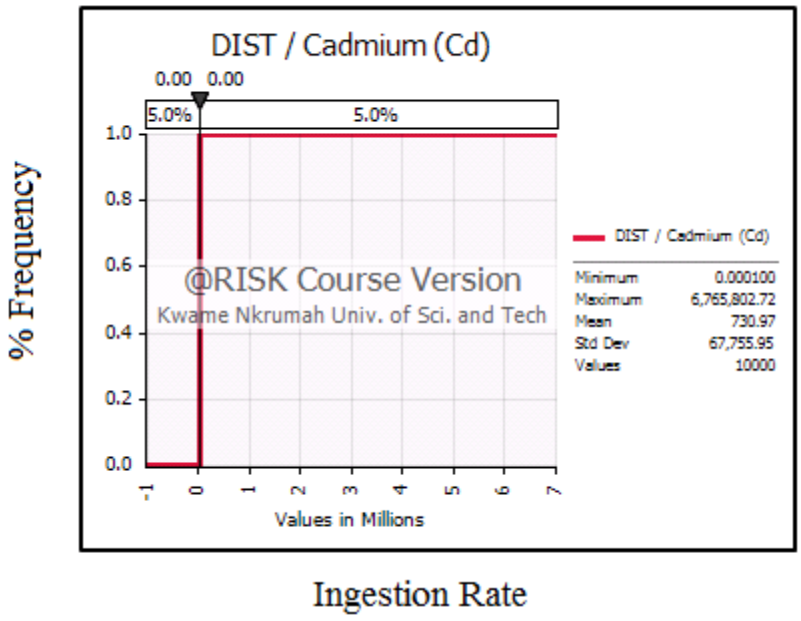

Figure 5: RiskPareto Distribution of mean cadmium intake (mg/kg).

4.4.3 Mercury

The daily amount was found to vary with minimum of 0.0001mg/kg and the maximum of +∞. The average concentration of mercury was found to be 0.00102mg/kg.

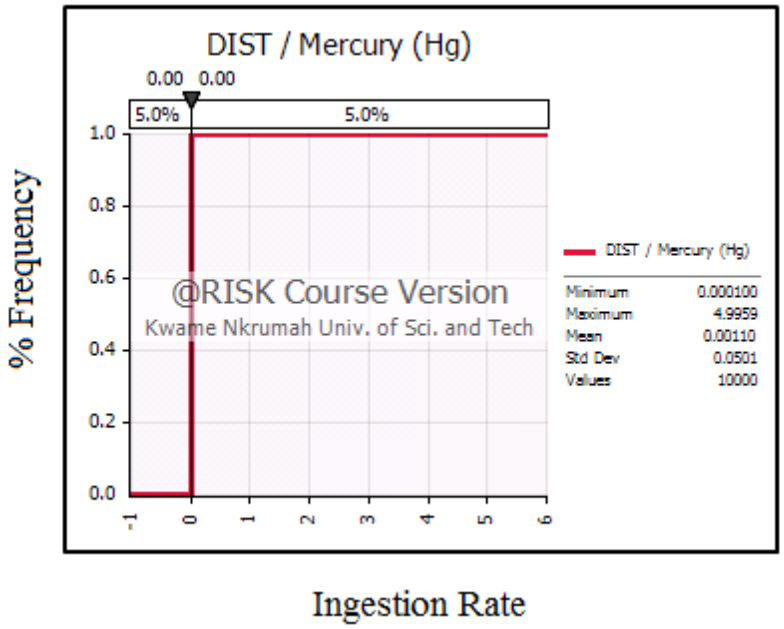

Figure 6: RiskPareto Distribution of mean Mercury intake (mg/kg).

A Pareto distribution was set on the amount of mercury, using the minimum arsenic content and the maximum arsenic content since the selection of tomato sauce to a consumer is random, hence any of the tomato sauce with an unknown specific amount will be given to a consumer.

4.4.4 Lead

The daily amount was found to vary with minimum of 0.04525mg/kg and the maximum of 0.60200mg/kg. The average concentration of lead was found to be 0.32362 mg/kg.

A uniform distribution was set on the amount of lead, using the minimum lead content and the maximum arsenic content since the selection of tomato sauce to a consumer is random, hence any of the tomato sauce with an unknown specific amount will be given to a consumer.

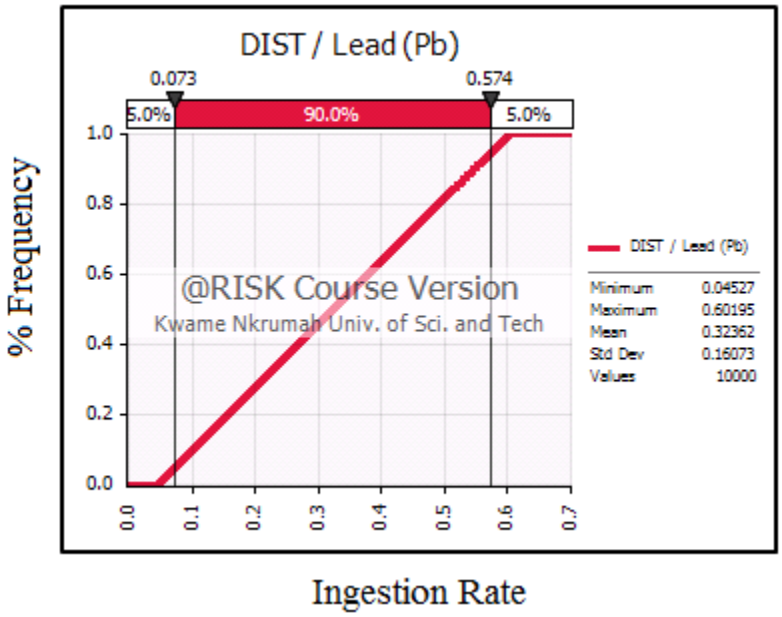

Figure 7: RiskUniform Distribution of mean Lead intake (mg/kg).

4.5 Chronic Daily Intake and Risk of Arsenic, Cadmium, Mercury and Lead

The reference dose, or RfD, of each metal, which is the intake or dose of the metal per unit body weight per day (mg/ kg/ day) that is likely to pose no appreciable risk to human populations, including such sensitive groups as children is shown on Table 4.

The Potency factors of each metal which is the reciprocal of the concentration of chemical measured in milligrams per kilogram of animal body weight per day, that is, 1/(mg /kg/day), or the risk produced by a lifetime average dose (AD) of 1 mg /kg/day is shown in Table 4.

The mean exposure concentration of As, Cd, Hg and Pb was used together with exposed population variables and the assessment determined variables to estimate contaminant

intake and Risk associated with the consumption of ready-to-eat tomato sauce in the Tamale Metropolis.

Table 4: Metal reference doses (RFD) and Potency factors (PF)

Metal	RfD(mg/kg-d)	Source	Potency Factor(mg/kg-d)	Source
Pb	1.4×10^{-4}	Oak Ridge[c]	0.085	TAC[f]
Cd	5×10^{-4}	IRIS[a]	6.3	Health Canada/US EPA[e]
As	3×10^{-4}	IRIS	1.5	Health Canada/US EPA
Hg	1.6×10^{-4}	Cal EPA[b]	*	

[a]Integrated Risk Information System,U.S. EPA; [b]California Environmental Protection Agency,U.S.; [c]Oak Ridge National Laboratory, U.S.; [d]The Agency for Toxic Substances and Disease Registry,U.S.;

[e]Health Canada/US EPA, http://www.popstoolkit.com/tools/HHRA/SF_USEPA.aspx.

[f]Toxic Air Contaminant document, Office of Environmental Health Hazard Assessment (OEHHA).

Figure 9, 10, 11 and 12 shows the distribution of chronic daily intake of arsenic ($1.07 \times 10^{-9)}$. cadmium (2.44×10^{-9}). mercury ($8.2 \times 10^{-10)}$ and lead (1.38×10^{-6}) whiles Figure 13,14, 15 and 16 show Arsenic (-0.00045), Cadmium (-0.000314), Mercury (-1.36×10^{-5}) and Lead (-1.2×10^{-5}) r risk associated with the consumption of ready-to-eat tomato by a subpopulation of Ghanaian adults living in Tamale over a one-year period.

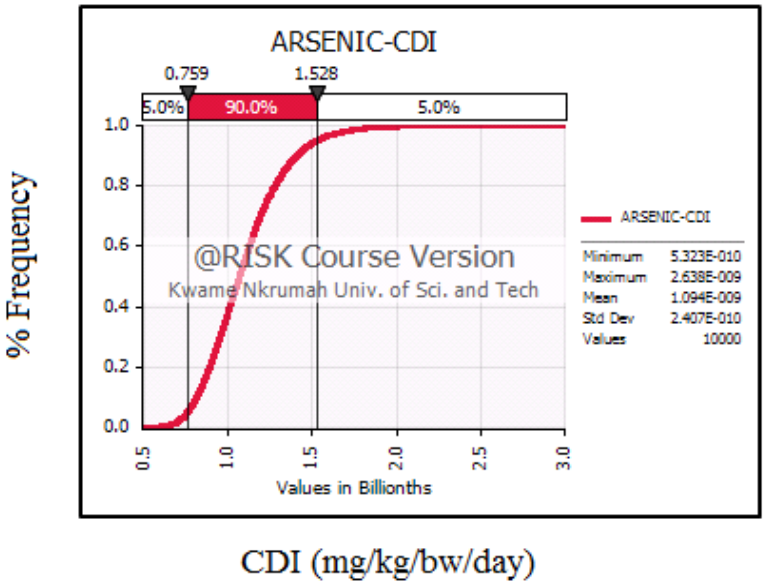

Figure 8: Chronic Daily intake of Arsenic (mg/kg/bw/day)

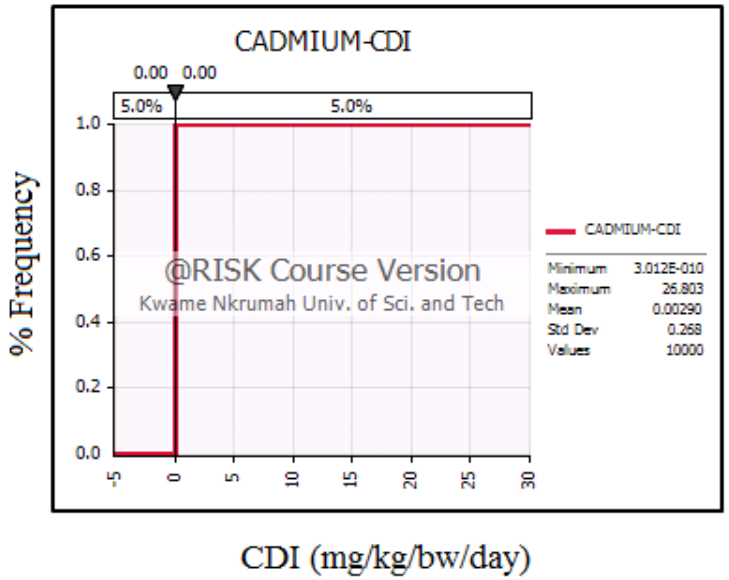

Figure 9: Chronic Daily intake of Cadmium(mg/kg/bw/day)

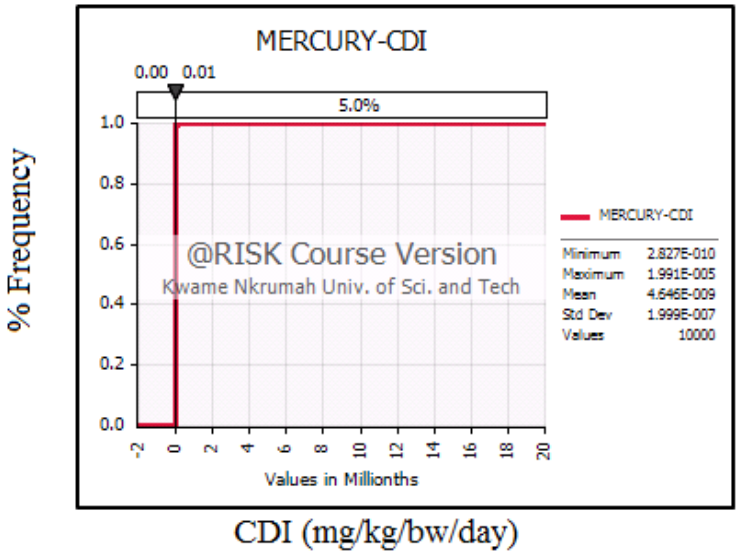

Figure 10: Chronic Daily intake of Lead (mg/kg/bw/day)

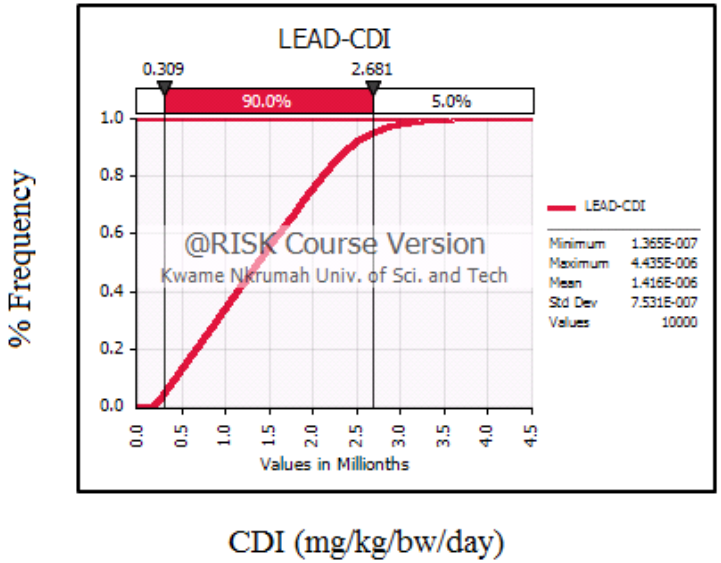

Figure 11: Chronic Daily intake of Mercury (mg/kg/bw/day)

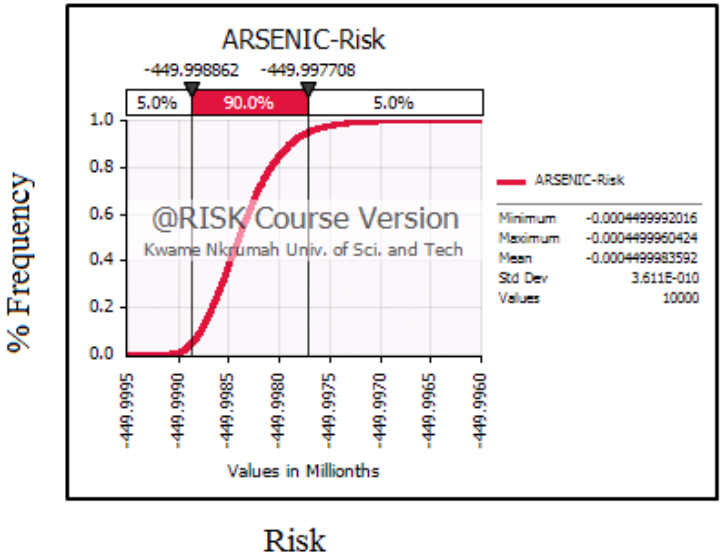

Figure 12: Risk distribution of Arsenic from consumption of ready-to-eat tomato sauce.

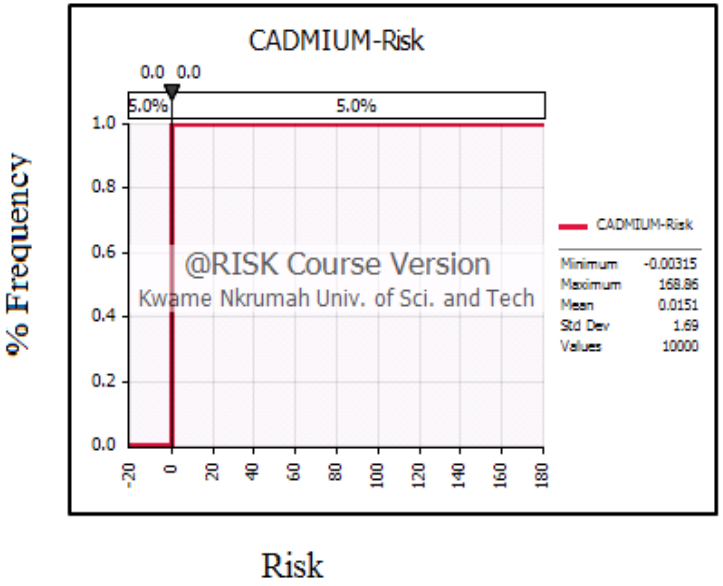

Figure 13: Risk distribution of Cadmium from consumption of ready-to-eat tomato sauce.

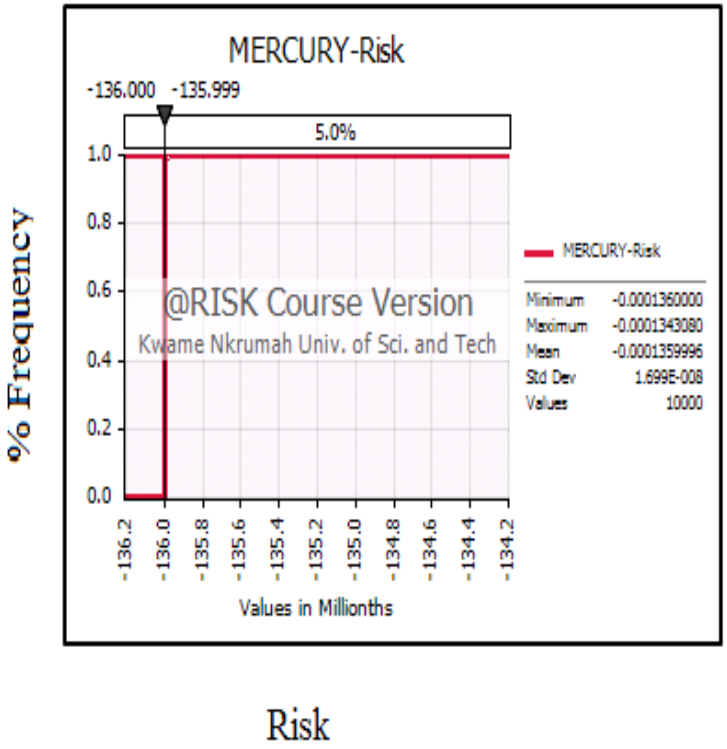

Figure 14: Risk distribution of Mercury from consumption of ready-to-eat tomato sauce.

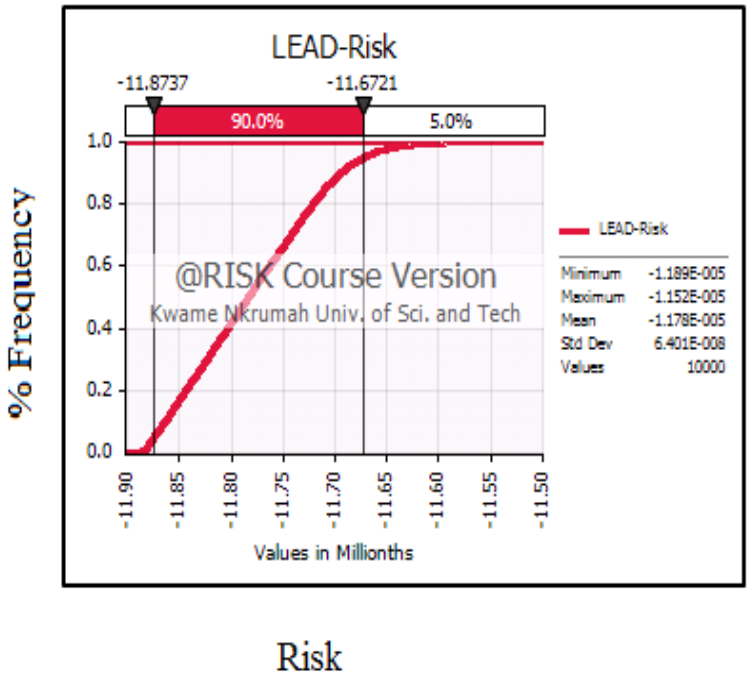

Figure 15: Risk distribution of Lead from consumption of ready-to-eat tomato sauce

4.6 Bacterial count of isolates in the food samples

The mean bacterial count of the isolates in the food samples were expressed as $\log_{10}$ cfu/g for easy computation. Food were classified as acceptable if the bacterial count was less than or equal to 5 $\log_{10}$ cfu/g.

Table 5: The comparison of total bacterial counts and contamination with Escherichia coli in the food samples tested.

	Samples No.	**The average of bacterial count (cfu/g)**	**The average of bacterial count($\log_{10}$cfu/g)**	**Positive cases of *Escherichia coli***
MILL A	3	$4.03 \times 10^6 \pm 1.23 \times 10^6$	6.7 ±0.12	1(33.3%)
MILL B	3	$2.1 \times 10^8 \pm 2.22 \times 10^8$	7.2±0.9	2(66.6%)
MILL C	3	$1.62 \times 10^7 \pm 1.01 \times 10^7$	6.9±0.4	2(66.6%)
MILL D	3	$2.32 \times 10^7 \pm 8.25 \times 10^6$	7.2±0.4	3(100%)
TOTAL	12	$4.80 \times 10^7 \pm 6.05 \times 10^7$	7.0±0.46	8(66.6%)

In terms of the comparison of the bacterial contamination among the four Mills investigated (Figure 16), the highest and lowest amount of contamination in the samples were respectively related to MILL B with an average of 2.1×10^8 Cfu/g and MILL A with the average of 4.03×10^6 Cfu/g. (Table 7).

Statistically, the difference between the total bacteria count in the samples were not significant ($p>0.05$)

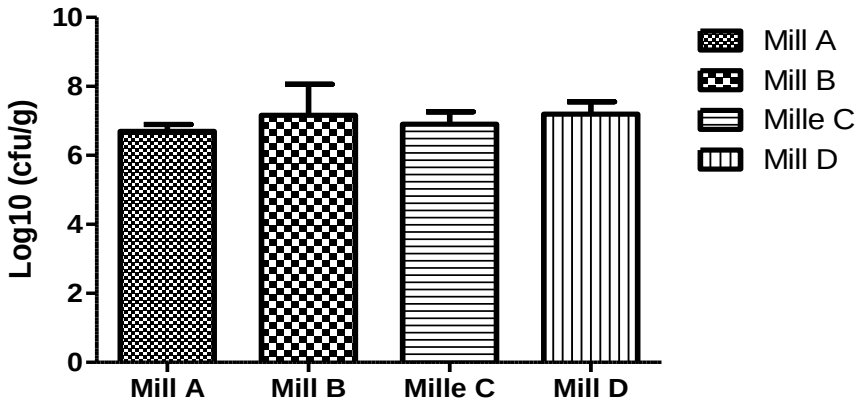

Figure 16: average total bacterial count in ready-to-eat tomato sauce from four different mills in the Tamale Metropolis.

Table 6: Predominant bacteria isolates from the various sources

Sample	**Isolates identified**
m1A	*Bacillus species*
M1b	*Bacillus species,*
M1c	*Bacillus species*
M2a	*Bacillus species*
M2b	*Bacillus species* and *Staphylococcus aureus*
M2c	Short rod *Bacillus species*
M3a	Short rod *Bacillus species*
M3b	*Bacillus species*
M3c	*Bacillus species*
M4a	*Bacillus species* and *E coli*
M4b	*Bacillus species* and *Staphylococcus aureus*
m4c	*Bacillus species* and *Staphylococcus aureus*

CHAPTER FIVE

DISCUSSION

The high concentrations of Pb could emanate from, feed, water source or the environment. Meanwhile, the concentrations of Arsenic and Mercury in the different sample sites were below all reference standards. The dietary intake of heavy metals from food sources is very crucial in order to evaluate the safety of food and consequently it effects on consumers. There was no available literature on the mean consumption of foods under study in Ghana.

Arsenic compounds are directly toxic to body system because after absorption of inorganic arsenic, this compound accumulates in the liver, spleen, kidney, lungs and gastrointestinal tract (Ismail, 2009).

From the results the concentration of Arsenic (0.0003 mg/kg) in the Tomato sauce where within tolerable limits in all sampling sites. However continuous exposure to (0.03 mg/kg) of Arsenic in foodstuff cause short term (nausea, vomiting, diarrhea, weakness, loss of appetite, cough and headache) could expose an individual to serious health effects. Arsenic has been classified by the International Agency for Research into Cancer (IARC) as a human carcinogen on the basis of increased incidence of cancers at several sites in people exposed to arsenic at work, in the environment or through their diet. However, arsenic is also more acutely toxic than other metallic compounds and was used in earlier times as a rodenticide, while continual low level exposure to arsenic is associated with skin, vascular and nervous system disorders (Food Safety Authority of Ireland, 2009).

The mean dietary intake of Arsenic from table 2 was 0.04µg/kg b.w/p/wk. The provisional tolerable weekly intake (PTWI) for Arsenic 15µg/kg body weight/week (JEFCA, 2009). The intake/kg body wt/wk was much lower than the PTWI as shown in table 2. The low % intakes of PTWI by the populace indicate low exposure to Arsenic form *ready-to-eat* tomato sauce.

Since all the concentration where high, it can be deduced the cadmium poses a significant health hazard to consumers. Sharma *et al*. (2009) recorded higher concentration of 1.96 mg/kg of cadmium in tomatoes more than that obtained in this study. According Sharma *et al*., (2009) high concentration of heavy metal such as cadmium in the vegetables like tomatoes may occur due to irrigation with contaminated water.

Jimoh *et al* (2012) also reported high levels of cadmium above the maximum limits. Studies conducted in Egypt by Radwan and Salama, (2006), on Egyptian fruits and vegetables, reported that heavy metals in tomatoes consist of Cd (0.01 ± 0.00 mg/kg) below the standard threshold.

The mean dietary intake of Cadmium (Table 2) show that the intakes (µg/kg bw/person/week) was 0.2.comparing the intakes with the PTWI guidelines of 2.5 µg/kg body wt/week stipulated by JEFCA. The intake of cadmium of the studied population was much lower than the PTWI as shown in Table 2.The low percentage intake of the PTWI by the studied population indicate low exposure to cadmium from *ready-to-eat* tomato sauce.

Mercury is more toxic than Cd and Pb (Abbas *et al*, 2010). The concentration of mercury exceeding the maximum permissible limit (0.03 mg/kg) in food and food stuff cause

serious health problems such as loss of vision, hearing and mental retardation and finally death occurs (WHO/FAO, 2004).

A study conducted in the industrial area of Huludao City, China by Na Zheng *et al*, reported that heavy metals in tomatoes consist of Hg (0.232 mg/kg), above the permissible limit.

The main concern in relation to the toxicity of mercury in the general population exposed to low levels of mercury in their diet relates to the potential neurotoxicity of organic forms of mercury, e.g. methyl mercury, in young children (EFSA, 2004). Mercury in its organic form can cross the placental barrier between the mother and the unborn baby. Epidemiological studies in exposed populations of humans and toxicological studies in animals have shown that this can result in a range of neurological disturbances from impaired learning to obvious brain damage.

The mean dietary intake of mercury (Table 2) shows that the intakes (µg/kg bw/person/week) was 0.01. Comparing the intake with the PTWI guidelines of 4 µg/kg body wt/week as stipulated by JEFCA. The intake of mercury of the studied population from *ready-to-eat* tomato sauce was lower than the PTWI as shown on Table 2. The low percentage intake of the PTWI by the studied population indicate low exposure to mercury from *ready-to-eat* tomato sauce.

Consumption of food containing lead is the major source of exposure for the general population. The concentration of lead exceeding the maximum permissible limit (0.2 mg/kg) in foodstuff can pose both short and long term deleterious effects. Short-term

exposure to high levels of lead can cause brain damage, paralysis (lead palsy), anaemia and gastrointestinal symptoms.

This implies that, the concentrations of lead in tomatoes are above the tolerable limit in most of the sampling sites. However, from the results obtained in this research work consumers of such vegetables are liable to lead toxicity.

Longer-term exposure can cause damage to the kidneys, reproductive and immune systems in addition to effects on the nervous system. The most critical effect of low-level lead exposure is on intellectual development in young children and, like mercury, lead crosses the placental barrier and accumulates in the foetus. Infants and young children are more vulnerable than adults to the toxic effects of lead, and they also absorb lead more readily. Even shorterm, low-level exposures of young children to lead is considered to have an effect on neurobehavioural development (Food Safety Authority of Ireland, 2009).

Raduan and Salama, 2006, who carried out similar studies in Egyptian fruits and vegetables, they reported that heavy metals in tomatoes consist of Pb (0.26 ± 0.09 mg/kg).A study conducted to ascertain the oil, micronutrients and heavy metals contents of tomatoes by Aryan Dermisbas, (2009) reported Pb concentration (0.43 ± 0.08 mg/kg).Another study conducted in the industrial area of Huludao City, China reported Pb (6.62 mg/kg).

The mean dietary intake of Lead (Table 2) show that the intakes (µg/kg bw/person/week) was 0.8. Comparing the intake with the PTWI guidelines of 25 µg/kg body weight/week

as stipulated by JEFCA. The intake of mercury of the studied population from *ready-to-eat* tomato sauce was lower higher than the PTWI as shown on Table 2.

The degree of toxicity of heavy metals to human being depends upon their daily intake. Heavy metals intake through consumption of vegetables consumed showed large variations. Our Chronic Daily Intakes for the heavy metals were below their respective reference dose as seen on Table 3. Since the RfD is used as a simple indicator of potential risk in practice. That is, the chronic daily intake is simply compared with the RfD, then, if the CDI is below the RfD, it is assumed that the risk is negligible for almost all members of an exposed population.

The cumulative frequency graphs below shows that, the risk of heavy metal ingestion via consumption of ready-to-eat tomato sauce was the same throughout the 10,000 simulations, hence the uniform risk for all consumers. Mercury has no potency factor, a reference potency factor for Lead (0.085) was used for the risk calculation, and this may be too high or low.

The cumulative probability of risk for Arsenic (-0.00045), Cadmium (-0.000314), Mercury (-1.36×10^{-5}) and Lead (-1.2×10^{-5}) as shown on figure 12, 13, 14 and 15 was in the negative indicating no significant risk. Radwan & Salama (2006) and Khan *et al*. (2008) have also observed no risk due to consumption of common foodstuff grown under waste water irrigated areas. In the present study, Cd is mainly responsible for causing human health risk. Zheng *et al*. (2007) have observed that daily intake of Cd was higher than the tolerable daily intake limit around Huludao Zinc Plant, thus causing a threat to the inhabitants of the area.

Bempah *et al* (2011) did report in their study of fruits and vegetables in the Ghanaian markets that heavy metals in fruits and vegetables did not pose any immediate risk to human health so far, but continues consumption of such fruit and vegetables even with moderate contamination level can accumulate in the receptors body and may prove fatal for human population in the long term.

For this risk assessment, the focus was placed on the variability of direct exposure due to time activity patterns of the population. Sources of variability were accommodated using Monte Carlo Simulation.

Microbial analysis was conducted on all samples to determine the microbiological safety (Table 5). The purpose of the analysis was to provide an indication of the types of viable microbes that were able to contaminate the products. The standard plate count (SPC), or viable count, is commonly used to indicate the microbiological quality of food.

The mean value of total bacterial count on the four different mills (Table 7) were 6.7 ± 0.12, 7.2 ± 0.9, 6.9 ± 0.4 and 7.2 ± 0.46 $\log_{10}$ cfu/g for MILLS A,B,C and D respectively. The overall mean average (n=12) was 7.0±0.46

Most of the samples tested in this study did not meet satisfactory level of microorganisms ie $>10^6$ cfu/g which is the recommended standard limit for bacterial count in *ready-to-eat* foods. Other works by Mensah *et al*. (2002) found a bacterial count of 6.3 ± 0.78 in salads sold on the streets of Accra. Likewise, Christison *et al*. (2008) also reported high bacterial prevalence in filled baguettes and salads. Bukar *et al.* (2010) in his study on ready-to-eat-foods in South Africa also found mean bacterial levels above the recommended standard.

The presence of *E. coli* in ready-to-eat foods is undesirable because it indicates poor hygienic conditions which have led to contamination or inadequate heat treatment

Meanwhile, of the 12 food samples tested, 8 samples (66.6%) contamination to *Escherichia coli* was confirmed, out of which 3 cases were related to Mill D ,2 cases to MILL B and C and 1 case to MILL A. Contamination of *ready-to-eat* tomato sauce with staphylococci is largely as a result of human contact. Their presence in the sample indicate time/temperature abuse likely to have occurred following improper handling. Levels of $\geq 10^4$ cfu are considered as potentially hazardous as foods with this level of contamination may result in food borne illness if consumed

Contamination can be minimized through good food handling practices and growth of the organism prevented through adequate temperature controls.

An unsatisfactory level of *B. cereus* in cooked foods generally occurs as a result of inadequate temperature control. The detection of high levels ($>10^3$ cfu per gram) of *B. cereus* should result in an investigation of the food handling controls used by the food business.

Levels of $\geq 10^3$4cfu per gram are considered potentially hazardous as consumption foods with this level of contamination may result in food borne illness. Other *Bacillus* species, such as *B. subtilis* and *B. licheniformis*, have also been associated with food borne illness and may also be tested for.

This study provides basic data on the distribution, prevalence, and levels of Bacillus sp. in selected retail products. Overall, 98% of the samples tested were found to have counts

of this pathogen ≥.6 log cfu/g, placing them in the unsatisfactory category for Bacillus sp. in ready-to-eat foods (Food Standards Australia New Zealand, 2001).

All of the sauce samples were of unacceptable quality due to pathogenic *Bacillus* spp. (*B. subtilis, B. pumilis, B. licheniformis*) at $\geq 10^5$ cfu/g. Results of the study is indicative for contamination and inadequate of hygienic conditions in production and processing of *ready-to-eat* tomato sauce. Ready-to-eat tomato sauce contaminated with pathogens such as *Escherichia coli* or with unacceptable levels of *S. aureus* or *B. sp.* are unsafe. They are considered to be injurious to health and/or unfit for human consumption. The results emphasize the need for good hygiene practices in handling these types of ready-to-eat products.

CHAPTER SIX

6.1 CONCLUSION

As and Hg show no possibility of detrimental effects on humans. These heavy metals, however, must be monitored to ensure that they stay at harmless levels. The other metals (Pb and Cd) are very close to if not above safe levels. The concentrations exhibited may not presently result in any deleterious or harmful effects however; further consumption of said metals, from any source, may have dangerous health effects. The consumption of ready-to-eat tomato sauce does not pose unacceptable risk to the population although the main exposure route is consumption of vegetables.

The growing rate of urbanization and the use of size reduction metal machinery coupled with the use of waste water on vegetable farms has resulted in increased levels of heavy metals and microbial contaminants in our foods. The bacterial count of all the samples collected were above the reference standards and thus pose a significant risk to consumers.

The result of this study however indicated that although the risk of consumption of locally milled ready-to-eat tomato consumed in the Tamale metropolis is low in the studied heavy metals

The dietary exposure analysis on the studied population revealed low exposure of these metals from locally milled ready-to-eat tomato. It can be concluded that inhabitants of the metropolis would be unlikely to experience major toxicological effects of the four heavy

metals studied. It therefore appears that they are not at any imminent health risk of excess exposure from locally.

To conclude, this study, in accordance with many others, the relative contribution of ready-to-eat tomato sauce on population exposure to significant health-related problems is small. But the study provides data and illustrates an approach that is useful for risk communication and management.

6.2 Recommendations

1.0 These findings demonstrate that ready-to-eat tomato constitutes a potential hazard microbiologically to human health. The isolation of E. *Coli* in ready-to-eat foods that are fully cooked is a good indicator of post-processing contamination or inadequate cooking. Therefore access to running water and health education to the vendors and millers on personal hygiene, food safety and proper disposal of waste would improve food quality thereby reducing food borne incidences.

2.0 Further research needs to be conducted on the actual consumption percentages of locally milled tomatoes and other vegetables. The research should also expand to look at different types of vegetables such as cabbage, carrot, lettuce, amarantus and onions since these could increase the risk of heavy metal consumption.

3.0 This occurrence of microorganisms in the samples indicate a need for improvement in the processing environment hygiene of street food. There is need to provide water, sanitary facilities and waste collection services, training programs and educating the street food vendors. A more in-depth study (multiple street foods prepared using ready-

to-eat tomato and other vegetables) and an upgrade of data regarding total consumption rates taking into cognizance different population groups would help in making strong statements concerning toxic and carcinogenic effects from exposure to Pb, Cd, As and Hg.

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APPENDICES

APPENDIX I

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

DEPARTMENT OF CROP AND SOIL SCIENCES

SOIL SCIENCE SECTION

REPORT OF ANALYSIS **DATE: 11TH JANUARY, 2014**

Sample id	←----------mg/kg---------->			
	Arsenic (As)	**Cadmium (Cd)**	**Mercury (Hg)**	**Lead (Pb)**
A1	0.0003	0.2407	0.0067	0.1088
A2	0.0002	<0.001	<0.001	0.1813
A3	0.0003	0.2257	0.0063	0.2487
B1	0.0002	<0.001	<0.001	0.2072
B2	0.0002	0.2758	0.0077	0.1761
B3	0.0002	<0.001	<0.001	0.2849
C1	0.0003	<0.001	<0.001	0.4559
C2	0.0003	<0.001	<0.001	0.5387
C3	0.0003	0.2758	0.0077	0.5595
D1	0.0002	0.0100	0.0030	0.3937
D2	0.0003	0.2407	0.0067	0.0881
D3	0.0002	<0.001	<0.001	0.4144

APPENDIX II

DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY

MSc FOOD QUALITY MANAGEMENT

Demographic, Anthropometric and Consumption Survey

FOOD FREQUENCY QUESTIONNAIRE

A. DEMOGRAPHY

1. What is your Religion?	**Christian**	**Traditional**		**Muslim**		**Others: Specify**		
Responses ➡								
2. What is your level of education?	**Primary**	**JHS**		**SHS**		**Tertiary**		**Others: Specify**
Responses ➡								
3. What is your Marital status?	**Single**	**Married**		**Widowed**		**divorced**		
Responses ➡								
4. How many people do you live with in your house?	**Alone**	**1**	**2**	**3**	**4**	**5**	**6**	**>6**
Responses ➡								
5. How many People do you eat with?	**Alone**	**1**	**2**	**3**	**4**	**5**	**6**	**>6**
Responses ➡								

B. ANTHROPOMETRY

1. How old are you?	<6	6-18	19-55	>55			
Responses ➡							
2. What is your Weight/kg?	**<40**	**40-45**	**46-52**	**53-60**	**61-70**	**>70**	
Responses ➡							

**** Please measurement would be taken on the scale provided***

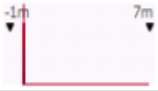
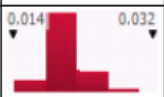
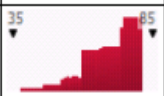
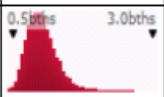
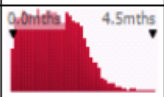
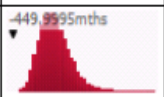
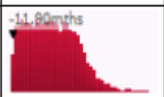
C. CONSUMPTION DATA

Please think about food you ate during the last month.

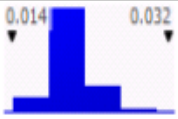
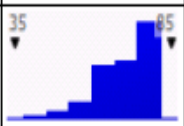
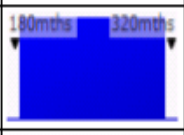
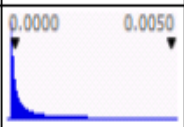
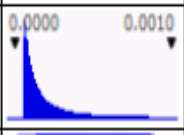
1 Do you take the pepper as part of your meal?	**Yes**	**No**				
Responses ➡						
2. How often do you take kenkey and pepper	**Never**	**Once per year**	**Once per month**	**once per week**	**Once per day**	**Others, Specify**
Responses ➡						
3. **What meal times do you eat kenkey and pepper?**	**Breakfast**	**Lunch**	**Supper**	**In between Breakfast and Lunch**	**In Between Lunch and Supper**	**Others, Specify**
Responses ➡						
4. what quantity of pepper do you take per meal	**Looks like A**	**Looks like C**	**Looks like C**	**Looks like D**	**Others, Specify**	
Responses ➡						

See sizes on chart

APPENDIX III

@RISK Output Results								
Performed By: ERNEST BONAH								
Date: 23 July 2014 11:24:12								
Name	Cell	Graph	Min	Mean	Max	5%	95%	Errors
DIST / Arsenic (As)	L14		0.000190921	0.000249955	0.000308994	0.000196806	0.000303094	0
DIST / Cadmium (Cd)	M14		0.000100003	730.9726	6765803	0.000113664	0.1786585	0
DIST / Mercury (Hg)	N14		0.000100003	0.001095603	4.99585	0.000104732	0.001491003	0
DIST / Lead (Pb)	O14		0.04527136	0.3236225	0.6019508	0.07307551	0.5741417	0
Cotact rate	R1		0.01500182	0.02100787	0.02998505	0.01696949	0.02540582	0
Body Weight	R9		40.00249	69.57128	79.99872	54.78122	79.20309	0
ARSENIC-CDI	V7		5.3229E-10	1.09387E-09	2.63842E-09	7.58647E-10	1.52787E-09	0
CADMIUM-CDI	V8		3.01244E-10	0.002897827	26.80312	4.75249E-10	7.6275E-07	0
MERCURY-CDI	V9		2.82725E-10	4.64563E-09	1.99056E-05	4.20322E-10	6.48603E-09	0
LEAD-CDI	V10		1.36479E-07	1.41615E-06	4.43531E-06	3.0892E-07	2.6813E-06	0
ARSENIC-Risk	V14		-0.000449999	-0.000449998	-0.000449996	-0.000449999	-0.000449998	0
CADMIUM-Risk	V15		-0.003149998	0.01510631	168.8565	-0.003149997	-0.003145195	0
MERCURY-Risk	V16		-0.000136	-0.000136	-0.000134308	-0.000136	-0.000135999	0
LEAD-Risk	V17		-1.18884E-05	-1.17796E-05	-0.000011523	-1.18737E-05	-1.16721E-05	0

APPENDIX IV

RISK Model Inputs						
rformed By: HAFIZ						
te: 23 July 2014 11:24:23						
Name	Cell	Graph	Function	Min	Mean	Max
Cotact rate	R1		RiskHistogrm(0.015,0.03,{0.0952,0.7177,0.1769,0.0102})	0.015	0.02100787	0.03
Body Weight	R9		RiskHistogrm(40,80,{0.68,2.7,7.43,22.64,24.66,41.89})	40	69.57133	80
tegory: DIST						
DIST / Arsenic (As)	L14		RiskUniform(0.00019091,0.000309)	0.00019091	0.000249955	0.000309
DIST / Cadmium (Cd)	M14		RiskPareto(0.4,0.0001)	0.0001	n/a	+∞
DIST / Mercury (Hg)	N14		RiskPareto(1.1085,0.0001)	0.0001	0.001021659	+∞
DIST / Lead (Pb)	O14		RiskUniform(0.045245,0.602)	0.045245	0.3236225	0.602