KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,

KUMASI COLLEGE OF SCIENCE

DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY

A STOCHASTIC DIETARY RISK ESTIMATE OF ACRYLAMIDE EXPOSURE IN COMMONLY EATEN FOODS AMONG CONSUMERS

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DECLARATION

I hereby declare that this submission is my own work towards the MSc and that, to the best of my knowledge, it contains no material previously published by another person, nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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DEDICATION

To God.

ACKNOWLEDGEMENT

To Mr Isaac W. Ofosu, my supervisor, who did more than supervise. You gave me confidence not only to graduate but also to live and a sense of direction when it was hard and I have hit bottom. Thank you. To Mrs. Ankar Brewoo for drowning my fears in your sound counsel. To Grace. To Pastors Kang-Milung your love made this possible. To Pastor Obed Obeng – Addae, my man of God.

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ABSTRACT

The thrust of this study was to estimate the risk of dietary consumption of acrylamide. Such a study is essential in order to monitor exposure levels and protect the safety of consumers. Stratified sampling was used to sample 89 food matrices from vendors in selected towns. Six hundred and twenty nine (629) consumers were interviewed using a semi-structured questionnaire in a one week dietary recall survey. Acrylamide quantitated using Thermo Scientific Mircro-Volume UV-Vis NanoDrop spectrophotometer. The analytical approach adopted in this work used the probabilistic method to account for the randomness of the data acquired. The findings from this study show a 50 % percentile acrylamide concentration in the foods analyzed as 1.56 mg/g, a 50 % percentile acrylamide exposure of 0.100 mg/kg-day, a modal cancer risk of $9x10^{-5}$ and modal neurotoxic risk -0.0008. The main conclusions drawn from this study are that there is no neurotoxic risk due to dietary acrylamide in the study area but there is a probable cancer risk of 9 out of 100,000 due to dietary acrylamide, which is

unacceptable compared to the $1x10^{-6}$ *de minimis* value. As such, this study recommends further investigation with a view to providing intervention towards the reduction of acrylamide levels to levels below 0.001mg/g in heat – processed commonly eaten foods.

LIST OF ABBREVIATIONS/ACRONYMS

AT	Averaging Time of the CDI
BW	Body Weight
CDI	Chronic Daily Intake
CR	Consumption Rate
CYP450	Cytochrome P450
DF	Degree of Freedom
DNA	Deoxyribonucleic Acid
EFD	Exposure Frequency and Duration
EFSA	The European Food Safety Authority
EPA	The United States Environmental Protection Agency
FDAG	The Food and Drug Authority, Ghana
FSA	The Food Standards Agency of the United Kingdom
GAMA	Glycidamide Mercapturic Acid (N-acetyl-S-(2-hydroxy-2-carbamoylethyl)cysteine)
GC-MS/MS	Gas Chromatography Tandem Mass Spectrometry
GSA	Ghana Standards Board
HC	Hazard Concentration
HI	Hazard Index
HPLC	High Performance Liquid Chromatography
HPLC-DAD	High Performance Liquid Chromatography with Diode Array Detector
HPLC-MS/MS	High Performance Liquid Chromatography Tandem Mass Spectroscopy
HQ	Hazard Quotient

HR-TOF-MS	High Resolution Time of Flight Mass Spectrometry
IARC	The International Agency for Research on Cancer
IRMM	The European Community Institute for Reference Materials and Measurements
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
KMA	Kumasi Metropolitan Authority
MoFA	The Ministry of Food and Agriculture, Ghana
PF	Potency Factor also known as slope factor
PSA	Primary Secondary Amine
rcf	relative centrifugal force
RfD	Reference Dose
SE	Standard Error
SEM	Standard Error of the Mean
USFDA	The Food and Drug Administration, a federal agency of the United States
VIA	Visual Inspection with Acetic Acid

CHAPTER 1

INTRODUCTION

1.1 Background

According to the Food Standards Agency (FSA, 2010) acrylamide is a chemical produced during the thermal processing of foods which naturally abound with sugars (having terminal carbonyl groups) and amino acids like asparagine, at elevated temperatures (> 120°C). Such processing and preparation methods as roasting, baking or frying therefore predispose starch-rich foods including potato, cocoyam, plantain, yam and maize to high levels of acrylamide when cooked longer at higher temperatures.

1.1.1 Acrylamide by the Maillard Reaction Pathway

Acrylamide or prop-2-enamide (CH_2=$CHCONH_2$), a water soluble crystalline solid, is formed by the Maillard Reaction (Shibamoto and Bjeldanes, 2009; Burcham, 2014). The Maillard reaction is known to enhance food flavor, aroma and savor (maltol), accounting also for desirable browning (melanoidins), but causing nutrient (amino acid) destruction in addition to acrylamide formation, the reaction rate being subject to factors as temperature, pH and time of processing.

1.1.2 Acrylamide toxicity and carcinogenicity

Acrylamide, although a veritable animal carcinogen and a confirmed human neurotoxin, is still tagged a “probable human carcinogen” (IARC, 1994), a probable human carcinogen being interpreted to mean that acrylamide's carcinogenicity has its manifestation in humans contingent, not only on its pharmacokinetics and pharmacodynamics, but also more importantly, on its concentration in the medium, the consumption rate of the medium, exposure rate and exposure duration of consumers (Pennisi *et al.*, 2013; JECFA, 2010; Leikin and Paloucek, 2008; IARC, 1994).

1.1.3 Acrylamide monitoring and its matrices

From the literature, a survey of substrates on which acrylamide studies have been conducted include mainly purely potato-based products, such as French fries and potato chips (Marchettini *et al.,* 2013; Sanny *et al.,* 2012; Knol *et al.,* 2009; Gokmen *et al.,* 2007b), a few non-potato based products, such as chestnuts and cereal foods (Karasek *et al.,* 2009; Senyuva and Gokem, 2006) and, fewer still, model systems such as asparagine and glucose simulates (Knol *et al.,* 2010; De Vleeschouwer *et al.,* 2008). Also from the literature it can be seen that potato, *Solanum tuberosum L.* is the most studied crop with reference to acrylamide. Acrylamide studies have been conducted on biologically cultivated potato (Marchettini *et al.,* 2013). Acrylamide studies have also been conducted on potato matrices in combination with other products such as roasted coffee, cookies and noodles (USFDA, 2003; Bortolomeazzi *et al.,* 2012; Anese *et al.,* 2010; Zhu *et al.,* 2008; Quayson Ayernor, 2007). Regulatory bodies including the European Food Safety Authority (EFSA) are currently assessing the level of exposure and toxicity (EFSA, 2012) in order to establish acrylamide enforceable regulatory limits.

1.1.4 Acrylamide risk assessment and the shift from deterministic to stochastic approach

Most studies in the area of risk assessment fall short of the stochastic standard, in that they usually estimate a central variable along with the confidence interval and/or standard deviation (Murniece *et al.,* 2013; Garcia *et al.,* 2015; Obon-Santacana *et al.,* 2013; Burley *et al.,* 2010; Schouten *et al.,* 2009; Wilson *et al.,* 2009). In short deterministic approaches to risk analysis although beneficial provide nothing more than a point data within a range of spread or error margin, which is then imposed on the population. As a result, stochastic methods are being used to enhance conventional toxicomedical models (Dourson *et al.,* 2013), dietary hazard exposure (Huybrechts *et al.,* 2011), acrylamide spectral analyses (Wang *et al.,* 2010), but only to a very limited extent, despite its immense advantages including utility and versatility. A stochastic method then, definitively,

specifies a set of outcomes along with the corresponding distribution of the chances of occurrence given a set of inputs or assumptions.

Despite the current enormous research output on acrylamide in the world, there has been little inquiries on acrylamide in Ghana and Africa generally (Quayson and Ayernor, 2007). This may be partly due to uncertainty associated with acrylamides carcinogenicity status, partly due to the perceived poor association of acrylamide toxicity to consumption, partly due to the absence of big business or government to finance such research and partly due to a general research focus on others research areas.

1.1.5 Sampling design in acrylamide assessment studies

Most of the risk assessment on dietary acrylamide reported no sampling design of the substrates used (Murniece *et al.,* 2013; Bongers *et al.,* 2012; Burley *et al.,* 2010; Larsson *et al.,* 2009b; Larsson 2009a; Obon-santacana *et al.,* 2015; Schouten *et al.,* 2009) largely because use was rather made of the national or regional acrylamide databases such as the European Community Institute for Reference Materials and Measurements (IRMM).

1.1.6 Acrylamide analytical quantitation

Acrylamide is quantitatively determined using high performance liquid chromatography tandem mass spectroscopy (HPLC-MS/MS) as recommended by USFDA (2003), pending further review (Bortolomeazzi *et al.*, 2012; Anese *et al.,* 2010; Karasek *et al.,* 2009), although gas chromatography tandem mass chromatography (GC-MS/MS) has also been used (De Vleeschouwer *et al.,* 2008; Zhu *et al.,* 2008). However, there are other unconsolidated methods such as high resolution time of flight mass spectrometry (HR-TOF-MS) and spectrophotometry in use (Bråthen and Knutsen, 2005; Quayson and Ayernor, 2007).

1.1.7 Ghanaian staples as a potential source of acrylamide toxicity

Most of the common foods in Ghana have the essential prerequisites for acrylamide formation, in particular carbohydrates and amino acids in varying degrees. For example *Arachis hypogaea*, (groundnut) is a legume but has a significant level of carbohydrate (13.3%) in addition to protein (27.6 %), while wheat flour contains 12.7 % protein and 63.9 % carbohydrate (Caballero, 2003). Also *Zea mays* (maize), *Musa paradisiaca* (plantain), *Dioscorea spp* (yam) and *Colocasia esculenta* (cocoyam) with 43.8 kg/head/year, 84.8 kg/head/year, 41.9 kg/head/year and 40.0 kg/head/year respective per capita consumption, are Ghanaian staples popularly consumed in various forms including roasted and fried forms (MoFA, 2011). They contain high amounts of starch and some amounts of protein. For example, maize kernel contains 71.5 % starch and 10.3 % protein and plantain 23.7 % starch and 1.1 % protein (Caballero, 2003). As such, they are predisposed to high acrylamide formation when processed at elevated temperatures.

1.1.8 Further research in acrylamide studies

Acrylamide matrices monitored are skewed towards potato and potato-based products because they are characteristically thermally processed, have high consumption rate and have high commercial value in advanced countries to the neglect of equivalent staples in not-so-advanced countries. For example, although staples like yam, plantain, maize and cocoyam in third world countries like Ghana, are heat processed, have high consumption rate and very high commercial value, little acrylamide studies have been conducted on them. Further research is therefore needed on foods in this category.

Also acrylamide risk assessments require further investigations into a representative food matrix sampling design. Further research is also needed to validate a standardized acrylamide quantitation protocols, although HPLC-MS/MS is currently preferred (USFDA, 2003). Studies conducted on

acrylamide are predominantly deterministic in nature and as such are poor representation when it comes to application to populations, thus, so highlighting the need for stochastic approaches to acrylamide risk assessment.

1.2 Problem Statement and Justification

Acrylamide is a neurotoxin and a probable human carcinogen (IARC, 1994) which may be formed during heat processing of maize, plantain, yam and cocoyam as well as fish and other such foods. Long cooking time increases the amount of acrylamide formed during the roasting, frying, baking or grilling. In addition to being formed at elevated temperatures in especially dry heat processing, the sale of roasted maize, plantain, yam and cocoyam for example requires that, they are kept heated for periods longer than required for their preparation on the fire as the vendor waits for customers (Brathen *et al.*, 2005).

Staples are relatively affordable, easily prepared, readily accessible and perceived to be comparatively prepared under hygienic conditions. For these reasons, patronage and consumption of staples as maize, plantain, yam and cocoyam as indigenous delicacies is increasing (MoFA, 2011). Hence, the need to assess the risk of dietary acrylamide exposure. As such, this study will attempt to provide estimates of the acrylamide levels in roasted snacks, exposure levels and the associated risk level in the population of the study area.

Furthermore, there is currently limited study and scarcely any regulatory monitoring of the acrylamide exposure levels in heat-processed maize, plantain, yam and cocoyam so far in many areas in Ghana (Personal Communication with FDAG/GSA). In view of the increasing consumption of these heat processed foods, there is grave concern for neurotoxicosis and/or the probable risk of cancer. The percentage of positive visual inspection with acetic acid (VIA) test

for cancer of the cervix for instance, is increasing. The percentage positives for VIA were found to be 1.9, 3.1, and 3.6 for 2009, 2010 and 2011 respectively according to the Ghana Health Service (2011). The incidence of cancer, hitherto generally unreported in Ghana, has been reported by the Ghana Health Service (2011) as a serious cause of death, treatment and outpatient visits in hospitals these days. Hence, the need for this study.

1.3 Main Objective

The main objective of the study was to determine the risk of acrylamide in consumed thermallyprocessed common foods.

1.4 Specific objectives

The specific objectives of the study were

1. To determine the acrylamide concentration of commonly consumed heat - processed foods using spectrophotometry.

2. To determine the chronic daily intake (CDI) of the said foods using Food Dietary Recall Survey.

3. To determine the risk of acrylamide using probabilistic risk assessment (PRA) with Monte Carlo simulation

CHAPTER 2

LITERATURE REVIEW

2.1 Acrylamide content in foods/regulatory levels

In addition to the type of food as a source of variation, acrylamide content in foods vary according to processing factors, storage and handling of the same food (Medeiros-Vinci, 2012). This is logical since food is a chemical repository with each food type, indeed each food cultivar, having a unique chemical composition, constituents of which may be differentially amenable to acrylamide formation. Foods with mainly carbohydrate content and some amount of amino acids

tend to produce a high level of acrylamide compared to those with mainly protein and some carbohydrate when processed at elevated temperatures. Eight protein foods analyzed had 22–116 ppb acrylamide content whereas eight cereals had 47–266 ppb and seventeen potato chips had 117–2764 16 ppb (Burcham, 2014). There is little information as to the acrylamide values for such products as yam, cocoyam, plantain, fried fish and other staples in less developed countries as Ghana.

Apart from food category type, acrylamide may vary according to cultivars or varieties of the same species. In particular, Marchettini *et al*. (2013) found high amount of fructose and glucose in Rossa di Colfiorito and Kennebec cultivars of potato relative to the Quarantina bianca genovese. The presence of free reducing sugars tends to increase the amount of acrylamide formation during food processing at elevated temperatures. As a result, they found high levels of acrylamide in the former relative to the latter. To reemphasize, acrylamide content increases with increasing temperature of processing up to a certain level. This is consistent with the fact that generally the rate of a chemical reaction increases with increasing temperature. Here, Bråthen and Knutsen (2005) found that a dry-processing temperature of 190 ^{o}C to 210 ^{o}C yields the highest acrylamide levels in starchy foods and cereals. Another processing parameter key to acrylamide content in addition to temperature is time of processing. Below a certain temperature limit, time of heating increases the acrylamide production. Beyond that limit, the heating time reduces the level of acrylamide formed by elimination reaction, the nature of this reaction being unclear Bråthen and Knutsen (2005). Conditions under which foods are stored before processing were found to affect the acrylamide level during processing. As a case in point, the sugar level, that results in higher production of acrylamide, is higher in stored potato depending on the storage temperature (Marchettini *et al*., 2013).

2.2 Acrylamide minimization and mitigation studies and methods

Anese *et al.* (2010) studied the physical removal of acrylamide from biscuits and potato chips by optimizing pressure, temperature and time in their processing because the area of acrylamide physical removal is yet to be fully explored. The advantage of physical removal being that it produces no adverse effect on the food product, being a post-processing step, it further poses no additional processing costs and by-reactionary interferences. Due precautions may have to be taken to minimize or eliminate any undesirable organoleptic effects likely to occur however. Such microscale explorations require replicate studies with large enough samples in the dynamic but real cooking contexts of industry though.

In practice, given equivalent concentrations of reducing sugars and asparagine in the starting material, different food vendors have differing levels of acrylamide in the final food product possibly because of the differing preparation methods and differing processing conditions and appliances used. There have been several enquires to optimize variables that affect acrylamide content in several foods especially potato, usually in a laboratory context. Current studies are therefore being directed at bringing such optimization studies in real food vending situations to understand the main factors that actually account for the variation of acrylamide among various categories of food vendors. For example, Sanny *et al.* (2011) looked at the activities of three groups: institutional, restaurant and fast-food food service providers, and how they prepared potato chips or French fries in order to find out the root causes of acrylamide variations for the same food product. Although such studies are complex and difficult to control, results obtained tend to be more reliable and readily industry applicable hence may be easily absorbed and adopted by industry stakeholders.

There is a real possibility to prevent carcinogenicity by reduction or altogether elimination of acrylamide whilst enhancing other desirable properties such as browning and flavor in the Maillard reaction simultaneously during food processing. One way of doing this is to exploit organic equivalents of inorganic compounds with known desirable effects on the Maillard reaction. In this direction, Wang *et al.* (2013) investigated the effect of a phosphoric acid ester of myo-inositol, (which is highly and readily available in the seeds of cereals for example) on both the acrylamide content and browning in the Maillard processing. The disadvantage here in using phytic acid, $C_6H_6(OPO_3H_2)_6$, is that it is also a chelating agent that easily mops up essential minerals, mainly of the divalent kind as Ca^{2+}, Mg^{2+} and Zn^{2+}, in foods as non-soluble derivatives, and hence makes them bio-unavailable.

Furthermore, other researchers reduce acrylamide content, whilst maintaining product quality specifications and properties, by using additives. In this direction Medeiros - Vinci *et al.* (2011) indicated that other aides to processing such as calcium, free amino acids and antioxidants also affect the acrylamide levels whether they are initially in the food substrate or added during processing. They are important considerations in optimizing to give a better representation of the complex nature of acrylamide interaction and formation.

In this regard, it must be noted that some vitamins, such as pyridoxamine and thiamine, have repressive effect on acrylamide formation in living organisms. However, the effect of vitamins on acrylamide formation during heat processing is novel and is now in an exploration stage. As a case in point, Zeng *et al.* (2009) looked at the effect of 15 vitamins and their effect on acrylamide formation in heat processing using both chemical simulates and actual food models. A success in this quest holds a promising potential, if real evidence is found as to vitamin inhibition of

acrylamide formation during heat processing, debarring any adverse by-reactions, to control acrylamide formation in a way that would also simultaneously enhance food nutrition levels and food value.

Manipulation of the starting material to manage the acrylamide level has also been explored as a real acrylamide minimization strategy that is very cost effective and that also enhances processing in that no undesirable by-reactions or products occur. Here, Gokmen *et al.* (2007a), whilst optimizing time and temperature of baking cookies also varied the dough formula with respect to the amount of various sugars and the pH of the dough to assess the effect on acrylamide formation.

2.3 Acrylamide determined in foods

From the literature, a survey of substrates on which acrylamide studies have been conducted are presented in table 2.1. It has been categorized into purely potato-based, non-potato based and model systems. It is evident from the table that potato, *Solanum tuberosum L*. is the most studied food crop with reference to acrylamide. Acrylamide studies have been conducted on biologically cultivated potato (Marchettini *et al.,* 2013), potato crisps (Knol *et al.,* 2009a; Kita *et al.,* 2007; Senyuva *et al.,* 2006), French fries (Pedreschi *et al.,* 2008; Sanny *et al.,* 2012, fried potato strips (Zeng *et al.,* 2009), potato chips and French fries as a combination (Gökmen *et al.,* 2007b). Acrylamide studies have also been conducted on potato matrices in combination with other products. For example, potato chips and biscuits (Anese *et al.,* 2010), potato chips along with biscuits and noodles (Zhu *et al.,* 2008). Even organically produced potato powder and conventionally cultivated potato powder have also been analyzed for acrylamide after heat processing (Carillo *et al.,* 2012). Similarly, studies have also been conducted on chestnut-based foods (Karasek *et al.,* 2009), cereal-based foods (Senyuva *et al.,* 2006), roasted coffee

(Bortolomeazzi *et al.*, 2012), cookies from dough (Gökmen *et al.*, 2007), Starch and cereals (Bråthen and Knutsen, 2005), sweetpotatoes and plantain (Quayson Ayernor., 2007a).

In all these studies, the trend could be observed that these studies were conducted in countries where the food products were commercially important, patronage and consumption was high and processing was essentially heat-based. To illustrate coffee studies were done in Italy, where there is an age-old culture of drinking coffee all day as a life style; whilst sweetpotatoes and plantain research was done in Ghana where it is a staple (Quayson and Ayernor, 2007).

Laboratory models systems have also been simulated and acrylamide investigations done on them. For instance, fructose and asparagine models (Knol *et al.*, 2010) as well as asparagine cum glucose models (De Vleeschouwer *et al.*, 2008) have been studied. Although not limited to it, these enquiries were done largely to elucidate the mechanism of acrylamide chemistry in foods and food processing.

Table 2.1: A survey of acrylamide matrices studied in the peer reviewed literature

Acrylamide substrate	Details	Researchers/references
Potato based	Biologically cultivated potato	Marchettini *et al.*, 2013
	potato crisps	Knol *et al.*, 2009b; Kita *et al.*, 2007; Senyuva *et al.*, 2006
	French fries	Pedreschi *et al.*, 2007; Sanny *et al.*, 2012
	fried potato strips	Zeng *et al.*, 2009
	potato chips and French fries	Gökmen *et al.*, 2007b
	Organic and conventional potato powder	Carillo *et al.*, 2012
Non-potato based	chestnut based foods	Karasek *et al.*, 2009
	cereal-based food	Senyuva *et al.*, 2006
Mix of Potato based and nonpotato based	potato chips and biscuits	Anese, *et al.*, 2010
	potato chips along with biscuits and noodles	Zhu *et al.*, 2008

	roasted coffee	Bortolomeazzi *et al.*, 2012
	Sweet potato and plantain	Quayson and Ayernor, 2007
Model systems	asparagine cum glucose	Knol *et al.*, 2010; De Vleeschouwer *et al.*, 2008

Note would have to be made here that, additionally EFSA (2012) has been monitoring acrylamide in ten main food groups including French fries, potato chips, pre-cooked potato chips, soft bread, breakfast cereals, biscuits and cookies, coffee and its substitutes, baby foods and other products since 2007. This is a more comprehensive effort in terms of matrices of acrylamide exposure study. There are limited studies on such African staples as cassava, groundnut, fish, cocoyam, yam and plantain thermally processed in the African context.

2.4 Analytical and mechanistic studies on acrylamide

Isotopic dilution analysis with HPLC tandem mass spectroscopy has been used to assess acrylamide in chestnut-based foods prepared by roasting, the advantages of isotopic dilution including high precision, high accuracy, minimal loss of analyte, non-use of calibration curves and quantitative separation were thus exploited (Karasek *et al.*, 2009).

Multiresponse kinetic simulation may hold a potential to explaining pre-Maillard steps in acrylamide formation in a way that captures the relative contribution of all substrates and byproducts, hence foster a comprehensive understanding of the underlying mechanism of acrylamide formation and elimination. Here Knol *et al.* (2009) have in particular, applied the multisresponse kinetic approach to a fructose and asparagine model and in this way may have better elucidated alternate pathways or obscure substrate model systems to acrylamide formation, minimization and elimination. However, this approach is not without reproach in as far as it is

cumbersome and too detailed as to drag especially, thus requiring it to be augmented with other approaches including mass and heat transfer mechanisms. Further regarding its limitation, the kinetic multiresponse model although better reflects the complexity of real food as against the artificial combinations of reactants in the laboratory, it mathematical expression is equally complex and not easily attained (Knol *et al.,* 2009). A more accurate mathematical construct is therefore required to capture acrylamide mechanism in complex actual foods as potato chips or French fries with reproducible and predictive rigor.

In their research, Agnieszka *et al*. (2004) investigated foods made with cereals along with starch that has been gelatinized for acrylamide levels with respect to temperature changes during processing and duration of processing. This is important since acrylamide formation is contingent on temperatures at 120 ^{O}C and above during processing. It is also expected that given temperature at a given point, the longer the product is kept in-process, the more acrylamide is expected to be formed by the Maillard reaction pathway. Also, their choice of using starch based foods in particular cereals was appropriate because they are attributed to be the category of foods with one of the highest exposure rates.

Acrylamide content and starting amounts of materials in simulated systems of asparagine and glucose were investigated by optimizing their relative amounts within the context of thermal treatment. In particular, De Vleeschouwer *et al.* (2008) looked at the mechanism of acrylamide formation and degradation with respect to the reacting species and their respective concentrations. Such studies derive their importance from providing clear information on the limiting reactants in acrylamide reactions, hence further furnishing the opportunity to evaluate which factors must be controlled in what way in heat-based food processing to reduce acrylamide formation.

2.5 Photometric method of acrylamide analysis

Efforts are being made to standardize computer based colorimetric methods in acrylamide determination in thermally processed foods. Consequently, attempts have been made particularly in this direction to establish a clear correlation between exterior image of food samples and acrylamide content (Gokmen *et al.,* 2007b). This will effectively enhance acrylamide determination in thermally-processed foods in two respects. Firstly, it would speed up acrylamide determination. Secondly, it would pave the way for acrylamide analysis automation.

2.6 Acrylamide management and organoleptic qualities

High fat content does not only reduce the organoleptic qualities of heat processed potato chips, it also has a potential to increase the acrylamide content with increasing high temperature (Kita *et al.,* 2007). This is because the fat content enhances acrylamide formation through the acrolein pathway, a mechanism by which fats are degraded. Studies have been conducted to optimize organoleptic qualities of potato chips along with temperature of the same in order to minimize or altogether eliminate acrylamide. In it, Kita *et al.* (2007) for example, investigated the thermal lability and oxidative lability of oil media in which potato chips or fries are processed and their joint or several effects on acrylamide.

2.7 Acrylamide in organic and conventional products

The debate has been raging on in the literature as to the veracity of qualitative and quantitative differences between organic foods and conventionally produced counterparts without a clear verdict other than that based on mores and ethics in favor of organic foods by a section of consumers and academic schools of thought. Against this background, Carillo *et al.* (2012) probed into the level of acrylamide in potato pulvil for selected varieties cultivated organically and conventionally. They looked at the effect of the temperature it took to dry, the duration of processing and the width of sliced potatoes on the physicochemical properties of organic potatoes

compared to conventional potatoes. It must be stated that it is a study that requires more than microscale laboratory simulated study, if it is to stand the test of real foods in the real world. Also, due consideration may have to be given to analyzing such foods with a stochastic approach to make it extrapolable to the general population.

2.8 Acrylamide research in developing countries

Despite the current enormous research output on acrylamide in the world, there have been little inquiries on acrylamide in Ghana and Africa generally. Quayson and Ayernor (2007) contend that the major research related to acrylamide was focused on cocoa, a cash crop and a major foreign exchange earner, despite the fact that Ghanaian staples are starch-based and prepared under high temperatures in dry cooking, making such foods highly susceptible to high acrylamide content. In the light of the obvious high exposure rate among the citizenry, the risk of acrylamide toxicity may be an issue worth investigating.

Acrylamide concentration is high in potato food products because potato is rich in acrylamide precursors: mainly carbohydrate and naturally-occurring asparagine. Also, the principal use of potatoes requires processing at elevated temperatures. Thus, creating a chemical system in which acrylamide is produced principally by the Maillard reaction. French fries and potato chips being main food products from potatoes are categorized as ‘junk’ among the convenience foods served by vendors. As a result, due to their generally high patronage, potato-based products have high exposure and consumption rate occasioning high level of research focus, inquiry and activity regarding acrylamide risk.

In some countries however, coffee with a similar acrylamide market profile as potato but relatively less acrylamide concentration, is ranked highest as the most important in terms of acrylamide

dietary exposure, principally, because it has a high exposure frequency, being consumed more often in a day than potato.

2.9 Stochastic and deterministic acrylamide risk studies

Murniece *et al.* (2013) assessed the risk of acrylamide toxicity among consumers of fried potato in Latvia. Like many other studies in this area, the assessment fell short of stochastic standard, citing the mean as a representative point data along with the confidence interval and/or standard deviation. This deterministic approach has serious shortfalls, including limits to its extrapolability (in it using only a handful of discontinuous data, data that is severely limited relative to the actual total population), limits to its representativeness of the distribution of acrylamide exposure or any dependent variable such as risk, in the population (it not capturing the probability of acrylamide toxicity), limits to its accuracy (in it curtailing the possibility of acrylamide toxicity of every member of the population to a single data point and so ignoring variability including sensitive subgroups of the population such as children, the very elderly, other vulnerable groups and resistant groups), and limits to its certainty in not accounting for the glaringly high level of uncertainty inherent in experimental units. In short deterministic approaches to risk analysis although beneficial provide nothing more than a point data within a range of spread or error margin. Popular of deterministic methods among food scientists include hazard quotient (HQ) which is an index of the exposure denominated by the reference dose, hazard index (HI) which is an aggregate of the HQ of individual chemo-hazards.

Correlational risk studies have also been done using odds ratio (Wilson *et al.,* 2009; Larsson *et al.,* 2009a,b) and hazard ratio (Garcia *et al.,* 2015; Burley *et al.,* 2010; Schouten *et al.,* 2009; ObonSantacana *et al.,* 2013) as standard methods in clinical trials or epidemiological cohort studies. These methods having almost gained standard status in medical research all churn out

point data, that essentially consist of an aggregate value representing the sample, and although they have unique advantages including being easy to calculate, flexible, highly used and appreciated by the medical profession (Spruance *et al.,* 2004), they are nonetheless deterministic, they are froth with the limitations that come with deterministic approaches to risk estimates itemized previously. As a result stochastic methods are being used to enhance conventional toxicomedical models (Dourson *et al.,* 2013) dietary hazard exposure (Huybrechts *et al.,* 2011), acrylamide spectral analyses (Wang *et al.,* 2010) but to a very limited extent, despite its immense advantages including utility and versatility.

A stochastic approach using Monte Carlo simulation for example works by iteratively generating output distributions and their corresponding probabilities from a corresponding distribution of the sample, and in this manner, create a more realistic representation of the model, capture the uncertainties across the sample population. In addition to the likelihood of each outcome, a Monte Carlo simulation spawns out probabilistic outcome graphs. Also Monte Carlo outputs could furnish probabilistic, correlational, sensitivity, optimization and scenario analyses. Furthermore, the builtin iterations enable a more realistic extrapolation of results of studies to the general population.

2.10 Food matrix sampling design in acrylamide risk assessments

Sampling biases can distort the results of acrylamide determinations and consequently, reduce the confidence that may be expressed in a given study. In this regard the EFSA defined sampling requirements and sampling report in its Europe-wide monitoring of acrylamide (EFSA, 2012).

Most potato substrates used in assessing the acrylamide level or examining the factors that influence the same during processing were sampled purposively from the market (Gokmen *et al.,* 2007b; Kita *et al.,* 2007; Knol *et al.,* 2010; Marchettini *et al.,* 2013; Pedreschi *et al.,* 2008; Quayson

and Ayernor, 2007; Senyuva *et al.,* 2006; Zeng *et al.,* 2009; Zhu *et al.,* 2008) as were similar studies involving chestnuts (Karasek *et al.,* 2009) and coffee brands from the roasting company or the market (Bortolomeazzi *et al.,* 2012). Model systems did not require any sampling procedure in that reactants were synthesized in the laboratory for the purpose of mechanistic studies altogether (DeVleeschouwer *et al.,* 2008; Knol *et al.,* 2010; Wang *et al.,* 2013). Organic varieties of potatoes were grown organically and conventional potatoes were also grown as such for the purpose of acrylamide and other assessment without any market sampling (Carillo *et al.,* 2012). Investigations looking at dough formulation on acrylamide formation were however prepared to ingredient specification requiring no sampling design (Gokmen *et al.,* 2007a). Some acrylamide studies however involved quota sampling to make provision for factors such as geography, processing methods (Sanny *et al.,* 2012a) and size (Anese *et al.,* 2010).

Most of the risk assessments of dietary acrylamide reported no sampling method of the substrates used or acrylamide content in the foods respondents consumed (Murniece *et al.,* 2013; Bongers *et al.,* 2012; Burley *et al.,* 2010; Larsson *et al.,* 2009b; Larsson2009a; Obon-santacana *et al.,* 2015; Schouten *et al.,* 2009) because use was made of the national or regional acrylamide databases such as the European Community Institute for Reference Materials and Measurements (IRMM). However, since most of these acrylamide risk studies were national or regional in scope, use of such reference databases may suffice, if they were up to date at the point in time they were used and due consideration was given to ensure equivalent food categories matched along with their preparation methods. It must be submitted that risk assessment of novel foods, previously unmonitored food types and monitored foods whose methods and processes of preparation, such as heat treatment and cold treatment, have changed or whose starting materials have also changed require actual determination of the current acrylamide content in the food matrix in order to be

accurate for obvious reasons. The latter is especially so since the content of acrylamide is determined by the method of preparation, and is influenced by the type, and in some cases the amount, of starting material. Doing so requires an effective sampling design. Methods of preparations are characterized by inherent variabilities which have direct effect on the estimated value of acrylamide in the food matrix for the study area.

The current method used by the EFSA (2012) is a combination of purposive sampling and probabilistic sampling, with the main sampling method being purposive sampling. It is expected however that a probabilistic sampling should be the mainstay when it comes to food sampling in order to ensure objectivity, reproducibility and possible generalizability.

Appropriately, the point of sampling prescribed for acrylamide food monitoring is the market and the point of production EFSA (2012). Excluding households, this way may not affect objectivity negatively, provided the foods being monitored are mainly commercially produced for public consumption, and all access points along the value chain, including significant household production points, are sampled. Foods for which preparation methods lend themselves to acrylamide formation and which have a high consumption rate and exposure rate but are not normally sold or are sold as much as are cooked and consumed in the privacy of their homes such as pertain to roasted snacks in the rural villages of Africa, may require special focus however, if the assessment is to be objectively representative.

2.11 Respondent sampling in acrylamide risk assessments

A major acrylamide risk assessment used cohort respondents from a computer registry cancer patients' database as a sampling frame, participation being subject to the respondents' willingness, state of health among other factors, and that being done 5000 respondents were randomly sampled

from 120,852 (Bongers *et al.,* 2012). Many cohort studies more or less follow this method of respondent sampling (Burley *et al*., 2010; Larsson *et al.,* 2009a; Larsson *et al.,* 2009b; Obónsantacana *et al.,* 2015; Obón-Santacana *et al.,* 2013), as it simultaneously achieves an ethical balance and theoretical foundation as well as some statistical rigor. The challenge here may be that since such studies have large sample sizes pooled from different countries, sampling methods may not necessarily be uniform.

2.12 Sample preparation methods

The sample preparation has generally included size reduction, centrifugation, homogenization and solid phase extraction operations (Anese *et al*., 2010; Bortolomeazzi *et al.,* 2012; Carillo *et al*., 2012; Sanny *et al*., 2012b; Marchettini *et al.,* 2013; Wang *et al*., 2013) using various devices where food matrices were researched on. Chemical model systems by their nature did not require any elaborate sample preparation (Knol *et al*., 2010) other than accurate preparation of the reactants in their right concentrations.

After the discovery of acrylamide's presence and potential toxicity in foods by Swedish scientists in 2002, research efforts were geared towards accurate quantitative determination of acrylamide in foods with high precision levels. As a result, research into analytical quantitation of acrylamide came to the fore. Spectrophotometry in its various forms have been used, for example (Quayson and Ayernor, 2007) as has time of flight mass spectroscopy (TOF-MS) been employed particularly for its sensitivity to analyze acrylamide (Bråthen and Knutsen, 2005). Although it has a high solvent use and it requires a lot of skills to operate, for the advantages of high accuracy, reproducibility and precision as well as its ability to preserve samples, high performance liquid chromatography (HPLC) in it various forms is used in acrylamide analysis.

2.13 Current understanding in acrylamide chemistry

Acrylamide also known *inter alia* as ethylene carboxamide or prop-2-enamide was discovered in foods, howbeit accidentally, by researchers in Sweden as more dangerous than its polymer (Burcham, 2014).

2.13.1 Structure and uses of acrylamide

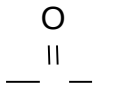
Acrylamide has an amide () and an alkene (C=C) functional group on the carboncarbon chain of three carbon atoms.

Figure 2.1 Acrylamide

In its polymeric form, as polyacrylamide, it has been used as a flocculating agent in treating water. However, since the acrylamide crystal is hydrophilous and soluble in water, it naturally has a propensity to leach in water purification and irrigation systems where it can enter the food chain, bioaccumulate and cause human toxicity. Also, polyacrylamide has found accepted and regular use in molecular biology by geneticists as an electrophoretic gel in genomic, transcriptomic and proteomic analysis.

Acrylamide's polymer is used as in the paper industry for sizing, coating and production of paper and paper products for such use as packaging. In molecular biology and paper production polyacrylamide is known to be generally safe. Similarly, polyacrylamide is used in the petroleum industry for such environment-friendly uses as removal of oil spillage (Leikin and Paloucek, 2008). A nitrofuran derivative in the form of 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide having acrylamide skeleton has been banned in foods as a definitive cause of cancer despite its antibacterial action (Burcham, 2014). So in its polymeric form, acrylamide has generally been found under regulatory

control to be safe. It is the monomeric form of acrylamide that has come to be the scare of carcinogenicity, neurotoxicity and reprotoxicity given it exposure level in heat processed foods.

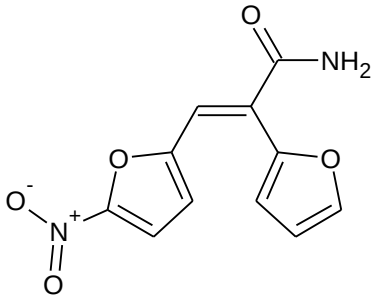


Figure 2.2: Structure of 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide

2.13.2 Acrylamide formation in foods

Acrylamide is formed as part of the Maillard reaction, the Maillard reaction being a mechanism of reactions with an amino group of an amino acid and glycosidic hydroxyl group from sugars as initial reactants and melanoidins as the end products (Hodge, 1953). Although the Maillard reaction generates desirable food attributes such food flavor, food color (browning), and antioxidants formation, it also causes deleterious effects including lysine degradation and acrylamide formation (Hodge, 1953). The Maillard reaction occurs in three stages: formation of Amadori product, Schiff base formation and acrylamide formation. In particular asparagine and a reducing sugar react by N-glycosyl conjugation to form a Schiff base, which on being decarboxylated, decomposes to produce acrylamide.

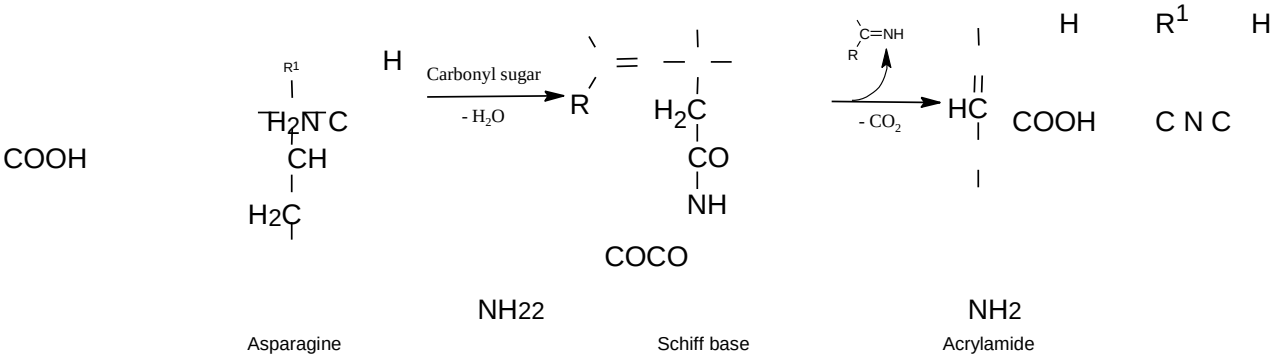


Figure 2.3: The Maillard pathway of acrylamide formation.

The reaction is plausible with asparagine and to a lesser extent glutamine because they already have amide moieties. Also, amino acids under normal culinary conditions undergo deamination and decarboxylation which are integral to the Maillard scheme. Furthermore, the amino acid must not be protein bound.

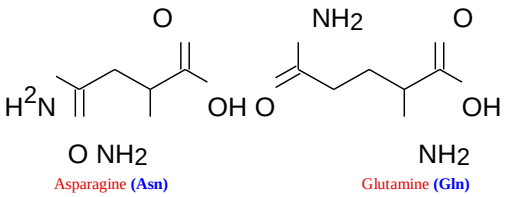


Figure 2.4: Asparagine and glutamine as amine precursors of acrylamide formation.

The hypothesized formation of acrylamide by the acrolein pathway cited for example by Shibamoto and Bjeldanes (2009) has largely remain a minor pathway of acrylamide formation if anything, in mainly carbohydrate foods but may be significant in mainly fat foods. The elucidations of the Maillard scheme of acrylamide formation is not only mechanistically and energetically plausible, but also has a huge data to support it over six decades.

2.13.3 Acrylamide exposure routes

Acrylamide enters the human biometabolism through the mouth, nose and skin (Shibamoto and Bjeldanes, 2009). Among these, the most important as a source of public health concern is the oral route through the consumption of heat processed foods rich in carbohydrate and protein in such common foods as coffee, cereals and bread. Skin exposure route relates with occupational acrylamide hazards among technicians for instance who do electrophoresis with acrylamide.

2.13.4 Acrylamide metabolism

Acrylamide is metabolized by mammals via conjugation and epoxidation as shown in figure 2.5. By its C_2 and C_3 double bond, acrylamide can conjugate with glutathione using glutathione-

Stransferase to form acrylamide mercapturic acid (N-acetyl-S-(2-carbamoyl-2hydroxyethyl)cysteine -S-oxide) (Shibamoto and Bjeldanes, 2009). This is a Phase II biotransformative metabolism. The mercapturic acid being more water soluble is then easily renally excreted.

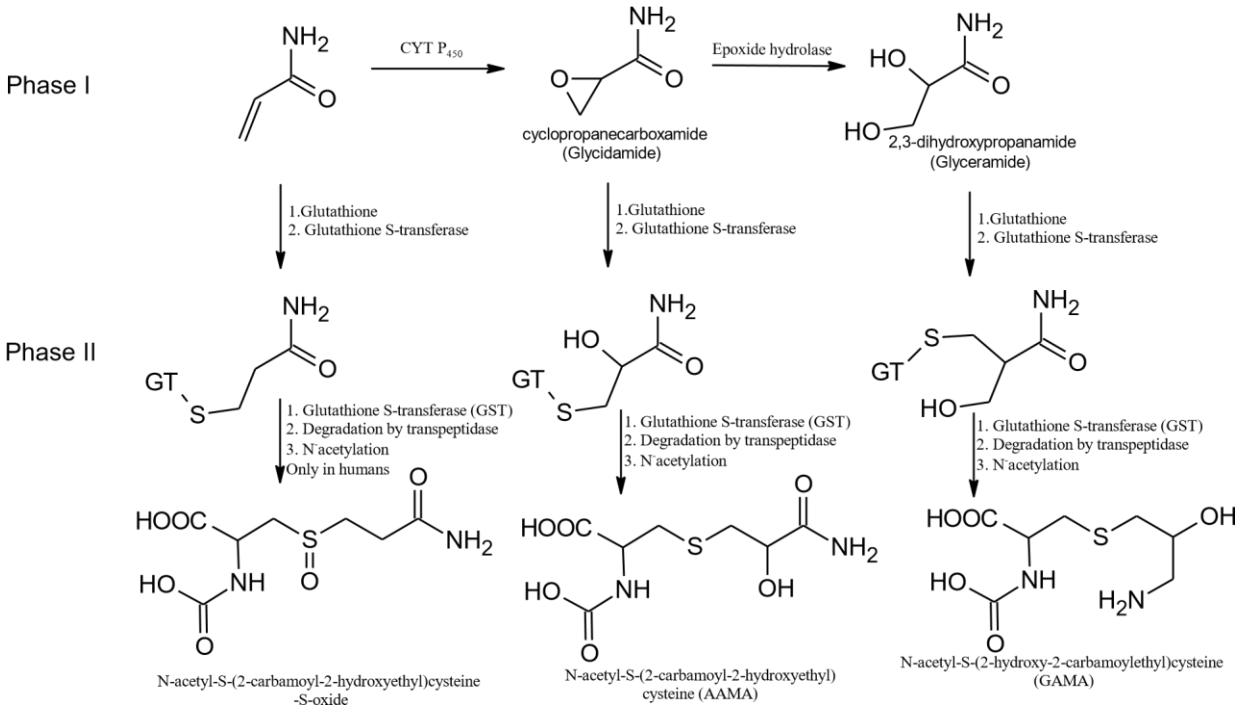


Figure 2.5: Mechanism of acrylamide metabolism adapted from Fuhr *et al.* (2006)

However, the major route by which acrylamide is metabolized is through epoxidation using cytochrome P450 (CYP450) to form glycidamide precedent to three possible pathways: formation of glyceramide (a phase I metabolite) catalyzed by epoxide hydrolase, formation of mercapturic acid (N-acetyl-S-(2-hydroxy-2-carbamoylethyl)cysteine or GAMA) and/or glycidamide left unmetabolized. The latter is thought to be the cause of acrylamide's genotoxicity (Shibamoto and Bjeldanes, 2009) even as both glycidamide and acrylamide can and do form adducts with DNA and as such prime suspects of mutagenicity and possible carcinogenicity. It must be qualified that

latter research indicates this rate is low. On ingestion, acrylamide trips to the stomach and is sent into the liver where hepatocytes metabolize it via routes mentioned above.

2.13.5 Acrylamide toxicity and health impact

Although acrylamide is consumed on a day to day basis in foods as common and accessible as coffee, French fries, cookies and bread, the human carcinotoxic effects is still unclear. Acrylamide is mainly phase II metabolized and is thought of as a possible carcinogen. This has led some researches to conclude acrylamide as a co-carcinogen in humans (Raju *et al.*, 2013). Acrylamide is a veritable animal carcinogen, confirmed human neurotoxin (Pennisi *et al.*, 2013) of mainly the peripheral nervous system with symptoms including ataxia, disorientation, paresthesia and tremors (acute toxicity) as well as sensorimotor and proprioceptive neuropathy (chronic toxicity) (Leikin and Paloucek, 2008). It is tagged as a "probable human carcinogen" by the International Agency for Research on Cancer (IARC) (JECFA, 2010; IARC, 1994). By probable human carcinogen, is meant that acrylamide's carcinogenicity having been proven in animals, its manifestation in humans is contingent on pharmacokinetics and pharmacodynamics including its concentration in the medium, the consumption rate of the medium, exposure rate and exposure duration of consumers. Regulatory bodies including the European Food Safety Authority, are currently assessing the level of exposure and toxicity (EFSA, 2012) in order to establish acrylamide enforceable regulatory limits.

To reiterate, both glycidamide, an acrylamide phase I metabolite, and acrylamide itself, can and do form adducts with DNA and as such, are linked to mutagenicity and possible carcinogenicity. However, for a number of reasons, the occurrence of carcinogenicity in humans is disputed. Whereas cancer/tumor formation to the consumption of acrylamide has been definitively established in animals, its occurrence in humans is inconsistent. Case-control studies to assess

acrylamide consumption and risk of several types of cancer, including oral cavity, pharyngeal, esophageal, laryngeal, colorectal, kidney, breast, and ovarian cancers, did fail to establish any correlation (Wilson *et al*., 2009; JECFA, 2011; Bongers *et al*., 2012; Stott-Miller, 2013), although a hormonal effect among women in post-menopause and a positive association between acrylamide intake and renal cell cancer has been reported (JECFA, 2011; Hogervorst *et al*., 2014). This is the main reason for the inability to designate acrylamide conclusively as a definitive human carcinogen.

Acrylamide in foods generally vary according to processing and agronomic factors including cooking time, processing temperature and variety. Whereas there are research output on the values of such products as potato and coffee that are produced in advanced counties, the same cannot be said of staples as plantain, yam, and others produced or prepared in less developed countries as Ghana. As a result, little is known as to the linkage of acrylamide related diseases including possible carcinogenicity in these parts of the world, even though there are concerns about rising incidents of cancer, for example in Ghana. Consequent to this, little effort has been made by way of mitigatory measures during food processing and agronomic practices in terms of acrylamide toxicity. Undergirding all this is a general dearth of research focus on acrylamide in less developed countries. Analytical and mechanistic studies have revealed a clear mechanism by which acrylamide formation occurs in foods by the Maillard reaction mainly in carbohydrate-rich foods processed at elevated temperatures and that high fat content diminishes desirable organoleptic properties in addition to enhancing acrylamide formation. Hitherto, acrylamide studies generally used deterministic models which limit the application of results of such studies to real populations. As such, there is evident need of stochastic methods that would enhance the level of accuracy of acrylamide studies to real populations. Whereas respondent sampling methods were clearly

reported, especially for cohort studies, acrylamide studies generally did not report matrix sampling method, this may have a telling effect on the reproducibility and accuracy of acrylamide studies and so a clear method of matrix sampling needs to be researched or applied especially for national and regional monitoring of acrylamide. Acrylamide analytical quantitation methods including HPLC–MS/MS, GC-MS and spectrophotometry are standard, although other validated methods like HPLC-DAD are used.

CHAPTER 3
MATERIALS AND METHODS
3.1. Materials
3.1.1 Matrices
The matrices for the study were purposively selected because of their high content of acrylamide precursors and therefore susceptibility to acrylamide formation (FSA, 2010) as baked foods: bread, meat pies, cakes, biscuits; fried foods: cocoyam chips, "buffloaf", fried yam, fried plantain and fish (*Trachurus japonicus*); roasted foods: yam, maize, plantain, cocoyam and groundnuts, as well as smoked/Grilled foods: smoked tuna (*Thunnus sp*).

3.2 Method
3.2.1 Study area
Kumasi is located at the centre of the Ashanti region midway of the country and serves as the region's capital and second largest city in Ghana. The population of 1.932 million is spread over an area of 254 squared kilometers. In the day however, the population of the metropolis swells to about 2.5 million due to the influx of traders and travelers from both neighboring regions and countries (Maoulidi and Ibrahim, 2010). Kumasi metropolis is made up of 10 administrative submetropolis.

3.2.2 Respondent sampling
Stratified sampling was used to select 2 towns per sub-metropolis using a list of the towns from the Kumasi Metropolitan Authority (KMA) as a sampling frame, and available and willing respondents were interviewed using a semi-structured questionnaire and taking their weight. Five enumerators assisted with the data collection. The enumerators were equipped with a one day training on the nature of the study, the structure of the questionnaire, survey techniques and questionnaire administration. Together with the researcher, they moved together in sampled communities at the same time to gather the data.

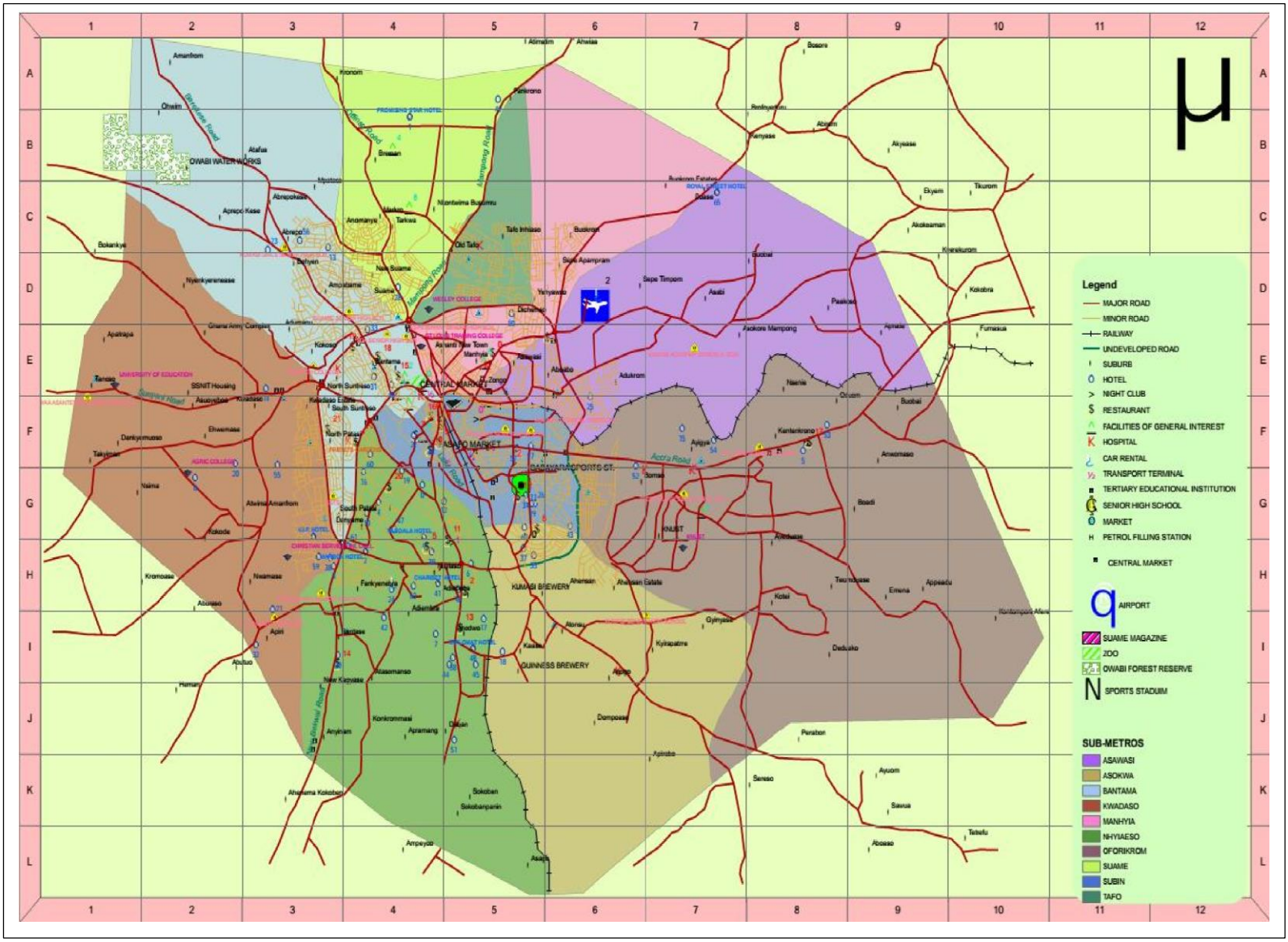


Figure 3.1: Map of Kumasi metropolis and its sub-metros

3.2.3 Matrix sampling

The matrix sampling was done subsequent to the town sampling (but separate from the respondent sampling) and in the sampled towns by going through streets and picking samples from every third vendor. Each sample was weighed and the price noted. In all, one hundred (100) samples were taken from five (5) sampled towns (using simple random sampling), each food item per town in the morning, afternoon and evening.

3.2.4 Analytical method

The extraction was achieved in the following way. To 1.0 g of sample in 50 ml centrifuge tube was added 4 g Magnesium Sulphate ($MgSO_4$) and 0.5 g Sodium Chloride (NaCl) followed by 5 ml of hexane($CH_3(CH_2)_4CH_3$). The mixture was vortexed for at least a minute and 10 ml of deionized water along with 10 ml acetonitrile added. The mixture then at 3450 relative centrifugal force (rcf) centrifuged for at least a minute. The supernatant liquid was then transferred into an injection vial. The cleanup was attained by the following procedure. On disposing the hexane floating layer and adding 1 ml methyl cyanide with 150 mg $MgSO_4$ and 50 mg primary secondary amine (PSA), the mixture was mixed for 30 seconds. After which the mixture was at 3450 rcf centrifuged for at least 1 minute and the supernatant transferred to a vial for injection. In order to quantitate acrylamide in the matrices, a stock solution of 10 mg/ml acrylamide was prepared and serially diluted to 0.0 mg/ml, 0.1 mg/ml 0.2, and 0.3 mg/ml acrylamide standards. Exactly 2 μl of each standard was then measured with a micro-pipette into the Thermo Scientific Mircro-Volume UV-Vis NanoDrop spectrophotometer, the equipment used to quantitate acrylamide in the food matrices. The quantitation was done by determining the absorbance of the standards at 220 nm. The results were plotted to obtain the standard calibration curve. The absorbances of the samples were then similarly measured and their concentrations determined from their respective absorbances on the standard calibration curve using the equation of the standard calibration curve

$$y = 4.098x + 0.015$$

with a coefficient of determination, R^2, of 0.9991.

3.2.5 Model specification

The @Risk software was used to fit the distribution of the hazard concentration (HC), consumption rate (CR), exposure frequency/duration (EFD), body weight (BW), and the acrylamide risk estimated using the empirical model:

$$\text{Risk of carcinogenicity} = \left[\frac{\text{HC} \times \text{CR} \times \text{EFD}}{\text{BW}} \times \frac{1}{\text{AT}}\right] \times \text{PF} \qquad \cdots\cdots\cdots\cdots \quad (1)$$

at 10,000 iterations and first order Monte Carlo simulation, where PF is the potency factor and AT, the average time. For neurotoxicity use was made of

$$\text{Risk of neurotoxicity} = \left[\left(\frac{\text{HC} \times \text{CR} \times \text{EFD}}{\text{BW}} \times \frac{1}{\text{AT}}\right) - \text{RfD}\right] \times \text{PF} \quad \cdots\cdots\cdots\cdots \quad (2)$$

Table 3.1: Model parameters and data sources, primary and secondary data used for the estimation of risk

Risk parameters	Data source
Hazard concentration(HC)	Food dietary recall survey
Consumption rate(CR)	Food dietary recall survey
Exposure frequency	365 days
Exposure duration	1 year
Body weight	Food dietary recall survey
Potency factor (PF)	0.5(mg/kg-day)$^{-1}$(IRIS, 2012) oral acrylamide PF for water)
Averaging time(AT) for carcinogenicity	70 years (Gerba, 1999)
Averaging time(AT) for neurotoxicity	30 years (Gerba, 1999)
Reference dose(RfD)	0.002mg/kg-day (IRIS, 2012) oral acrylamide slope factor for water)

This models assume that, and for that matter limited to the extent that, the oral RfD and PF of water is the same as that of food and that there is total absorption of ingested acrylamide (Gerba, 1999). The RfD and PF are determined essentially on the basis of route and hazard. The assumptions are justified on the basis that because both water consumption and food consumption use the oral route, their RfD and PF should be identical in either case for the same hazard, acrylamide.

3.2.6 Data analysis

The data was tabulated in Microsoft Excel 2013 and analyzed using Palisade @Risk 2015 software to simulate acrylamide risk in the roasted snacks using the Monte Carlo simulation. The data was entered in excel spreadsheet columns as hazard concentration, consumption frequency, mass of medium consumed, exposure frequency, exposure duration and body weight. Each column of data

was fitted to an appropriate or best fit distribution using the @Risk "distribution fitting" function specifying whether it is discrete or continuous and writing the aggregate output to a designated cell. Each value so generated was added to a unit excel cell using the "add output" tool and simulated at first order and ten thousand iterations. A construct of the empirical model was built using Excel formulas into which the model parameters were referenced to determine the risk using the potency factor value from IRIS (IRIS, 2010). The risk value so generated to cell was "added to output" and simulated at first order and ten thousand iterations. Excel reports, including graphs, of the outputs were then extracted as results for discussion.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Acrylamide concentration

The mean, mode, 5 %, 50 % and 95 % percentile acrylamide content in mg/g was found to be 0.48 mg/g, 0.44 mg/g, 0.15 mg/g, 0.47 mg/g and 0.82 mg/g respectively for "buffloaf" (Table 4.1). All these values exceed the 0.001mg/g indicative value for potato crisps from the EFSA (2012).

Table 4.1: Sample size(N), mean, mode, 5 %, 50 % and 95 % percentile values of acrylamide in mg/g of foods sampled in the study, including fitted distribution.

Matrix	N	Mean	Mode	5% Percentile	50% Percentile	95% Percentile	Fitted Distribution
"Buffloaf"	6	0.4756	0.4402	0.1475	0.4679	0.8211	Triangular (0,0.4423,0.98444)
Fried plantain	8	1.2964	0.5554	0.2961	1.1784	2.6757	Triangular (0,0.521,3.3683)
Tea bread	7	1.3092	0.4036	0.2661	1.1789	2.7835	Triangular (0,0.4018,3.5257)
Chips	5	1.3382	0.4114	0.2711	1.2050	2.8468	Triangular (0,0.4081,3.6066)
Fried Fish (*Trachurus japonicus)*	6	1.5775	0.0607	0.1434	1.3892	3.6393	Triangular (0,0.0512,4.6812)

Fried yam	7	1.6529	0.1999	0.2117	1.4640	3.7271	Triangular (0,0.185,4.7737)
Roasted cocoyam	6	1.6962	0.2940	0.2573	1.5083	3.7682	Triangular (0,0.2751,4.8134)
Roasted yam	6	1.6962	0.2941	0.2572	1.5082	3.7678	Triangular (0,0.27513,4.8134)
Smoked Tuna (*Thunnus sp*)	11	1.7925	0.3179	0.2784	1.5951	3.9720	Triangular (0,0.3062,5.0714)
Malt n Milk Biscuit	7	1.9541	0.5334	0.3673	1.7520	4.2177	Triangular (0,0.5039,5.3585)
Roasted groundnut	7	2.0775	0.2950	0.2798	1.8421	4.6642	Triangular (0,0.2615,5.9709)
Roasted plantain	9	2.3372	3.1552	0.2336	2.3370	4.4406	Uniform(0,4.6744)

The mean, mode, 5 %, 50 % and 95 % percentile acrylamide content of chips were 1.34 mg/g, 0.41 mg/g, 0.27 mg/g, 1.21 mg/g and 2.85 mg/g in that order, these values all are much higher than the 0.001 mg/g indicative value for potato crisps. Fried fish (*Trachurus japonicus*)values for acrylamide concentration were found to be 1.58 mg/g, 0.06 mg/g, 0.14 mg/g, 1.39 mg/g and 3.64 mg/g for the mean, mode, 5 %, 50 % and 95 % percentile respectively (Table 4.1). They also, exceed the 0.001 mg/g indicative value for potato crisps. In the case of fried plantain, the mean acrylamide content of 1.30 mg/g was determined, the mode was 0.56 mg/g, the 5 % percentile was 0.30 mg/g, the median value was 1.18 mg/g and the 95% was 2.68 mg/g which was also higher than the indicated value for crisps of potato . For fried yam, the corresponding values were 1.65 mg/g for the mean, 0.20 mg/g for the mode, 0.21 mg/g for the 5 % percentile, 1.46 mg/g for the 50 % percentile and 3.73 mg/g for the 95 % percentile. The Malt and Milk biscuit had 1.96 mg/g mean acrylamide content, 0.53 mg/g modal acrylamide content, 0.37 mg/g 5% percentile acrylamide value, 1.75 mg/g median acrylamide value and a 95^{th} percentile acrylamide value of 4.22 mg in each gram of the sampled biscuit. For every gram of roasted cocoyam, the mean acrylamide content was 1.70 mg, the modal acrylamide value was 0.29 mg, the 5^{th} percentile acrylamide content was

0.26 mg, the median acrylamide content was 1.51 mg and the 95th percentile acrylamide content was 3.77 mg. In roasted groundnut, the study found the acrylamide content to be 2.08 mg mean value, 0.0.30 mg modal value,0.28 mg 5th percentile value, 1.84 mg median value, 4.66mg mg 95th percentile value for each gram of the matrix. The roasted plantain analyzed gave the following results: 2.34 mg/g for the mean, 3.16 mg/g for the mode, 0.23 mg/g for the 5th percentile, 2.34 mg/g for the median and 4.44 mg/g for the 95th percentile. About 1.70 mg/g mean value, 0.30 mg/g modal value, 0.26 mg/g 5th percentile, 1.51 mg/g median value and 3.77 mg/g 95th percentile value was obtained for the levels of acrylamide in roasted yam. In smoked tuna (*Thunnus sp*) the acrylamide content was found to be distributed as 1.80 mg/g mean,

0.32 mg/g mode, 0.28 mg/g 5th percentile, 1.60 mg/g median, 3.97 mg/g 95th percentile. The concentration of acrylamide in tea bread ("tea bread") were 1.31 mg/g mean, 0.40 mg/g mode, 0.27 mg/g 5th percentile, 1.18 mg/g median, 2.78 mg/g 95th percentile. All the acrylamide concentration values, including the 5th percentiles, for all the matrices were found to be higher than the 0.001 mg/g indicative value for potato crisps [(33)]. In fact they were all higher than the maximum value for potato crisps, 0.0045 mg/g reported by the EFSA (2012). Any food product having acrylamide level at or above the "indicative value" according to EU specifications, requires further examination, provided such food products are not for home cooking. However, indicative values are not regulatorily enforceable on food producing firms and services companies and as such food products that breach the indicative values call for mandatory in-depth investigation of acrylamide in the context where they were produced or processed for public consumption on a case by case basis precedent to any regulatory action.

On the contrary, findings from the Swedish national studies indicate these acrylamide concentrations from the study are comparatively low (Svensson *et al*., 2012). The least acrylamide

concentration obtained was 0.03 mg/g and the maximum was 2.3 mg/g for products including cookies, breakfast cereals, bread, biscuits, snacks, potato products and coffee in Sweden.

The food matrix with the least mean acrylamide concentration was “buffloaf” yielding acrylamide concentration of 0.48 mg/g whereas roasted plantain had the highest with an acrylamide concentration of 2.34 mg/g among the food matrices studied. In terms of mode, fried fish had the lowest acrylamide concentration with a value of 0.061 mg/g whereas the roasted plantain had the highest with 3.16 mg/g acrylamide concentration. Roasted plantain had the highest median value (2.34 mg/g) whereas the “buffloaf” had the least (0.47 mg/g). The results show that the levels of acrylamide in terms of mean and median all present roasted plantain as having the highest acrylamide value and “buffloaf” the least.

4.2.1 Acrylamide concentration and processing methods

Now, a look at the effect of processing on the mean acrylamide content of the food matrices. Tukey's multiple comparisons test on acrylamide concentration in mg/g across processing methods yielded the above results in Table 4.2. At 95 % confidence interval, it could be seen that the differences in means were found significant for all the processing methods compared.

Table 4.2: Tukey's multiple comparisons test on acrylamide concentration in mg/g across processing methods for all food matrices.

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	q	DF	95% CI of diff.
Baked vs. Fried	1.599	1.419	0.1804	0.01594	16.01	39996	0.1395 to 0.2214
Baked vs. Grilled	1.599	1.793	-0.1934	0.01594	17.16	39996	-0.2344 to -0.1525
Baked vs. Roasted	1.599	1.88	-0.2811	0.01594	24.94	39996	-0.3220 to -0.2401
Fried vs. Grilled	1.419	1.793	-0.3739	0.01594	33.17	39996	-0.4148 to -0.3329
Fried vs. Roasted	1.419	1.88	-0.4615	0.01594	40.95	39996	-0.5025 to -0.4206
Grilled vs. Roasted	1.793	1.88	-0.08767	0.01594	7.778	39996	-0.1286 to -0.04672

As such, it can be inferred indicatively that roasting matrices yielded the highest acrylamide (1.88 mg/g), followed by grilling (1.79 mg/g). Baking was ranked third with acrylamide concentration

mean of 1.60 mg/g whilst frying yielded 1.42 mg/g as the least acrylamide producing method of processing.

Table 4.3: Rank of mean acrylamide levels of matrices by processing method.

Processing method	Mean/mg/g	Rank
Roasted	1.88	1
Grilled	1.793	2
Baked	1.599	3
Fried	1.419	4

The level of acrylamide depend principally on temperature at which the matrix is processed, followed by processing time and the level of reducing sugars in the matrix. Sanny *et al*. (2012a) for example, found it to be so for frying. They concluded that acrylamide concentration for (fried) French fries varies across service establishments according to processing practices. As such, it may be said of roasting that it is being done at a higher temperature for longer periods and that this accounts for the relatively high acrylamide content. Similarly, the underlying factors of low frying temperature and/or for shorter durations account for it being least in acrylamide yield. That the level of acrylamide in roasting is highest may plausibly be explained by the insight that the vendors of roasted foods in the study area kept their roasted products on fire after roasting is done to keep the food hot while waiting for customers. Grilled, fried and baked foods on the other hand are usually far removed from their heat sources after processing is done. For example turnovers, locally known as meat pies, and biscuits are usually sold without any heating once baking is done. Consistently, grilled foods are generally also heated after processing is done, but not as roasted foods. For example, tuna may be sold without heating after the initial grilling is adequately done. However it is heated occasionally to prevent spoilage. That may explain it being ranked second. Fried foods in the study area are generally removed from the processing heat source, and not heated again, once cooking is done. Hence, possibly explaining its low acrylamide level compared to roasting, grilling and baking.

It must be stated given the variability in matrix, a strict basis for comparison may not be justified, unless studies were conducted using the same matrix to ascertain the level differential in acrylamide with each processing method.

4.2.2 A comparison of acrylamide concentration in fried and roasted foods.

A look at the effect of processing on the acrylamide concentration given the same food matrix should bring into sharp focus the indication for the rank above. At 95 % confidence interval, there is a significant difference between the means of acrylamide in the roasted and fried plantain, with that of the roasted (1.30 mg/g) being higher than that of the fried (2.34 mg/g).

Table 4.4: Comparison of the means of acrylamide for roasted and fried plantain, and for roasted and fried yam using unpaired t-test with Welch's correction

Roasted plantain vs. Fried plantain	
P value	< 0.0001
Significantly different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
Welch-corrected t, df	t=67.62 df=15517
Mean ± SEM of Roasted plantain	2.337 ± 0.01349 N=10000
Mean ± SEM of Fried plantain	1.296 ± 0.007402 N=10000
Difference between means	-1.041 ± 0.01539
95% confidence interval	-1.071 to -1.011
Fried yam vs. Roasted yam	
P value	0.0056
Significantly different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
Welch-corrected t, df	t=2.773 df=19998
Mean ± SEM of Fried yam	1.653 ± 0.01104 N=10000
Mean ± SEM of Roasted yam	1.696 ± 0.01104 N=10000
Difference between means	0.04328 ± 0.01561

95% confidence interval	0.01268 to 0.07388

Similarly, there is a significant difference between acrylamide concentration means of the fried yam and roasted yam, with that of the roasted yam (1.70 mg/g) being higher than that of the fried yam (1.65 mg/g). The explanation given for the high level of acrylamide in roasted foods is applicable here.

The probability density distribution of acrylamide in buffloaf is almost symmetrical (skewness value of 0.1). Hence, the mean and the mode are very close. The distribution is also unimodal. Hence, the mode may be relied upon as a typical value. All the other matrices had similarly symmetrical distributions.

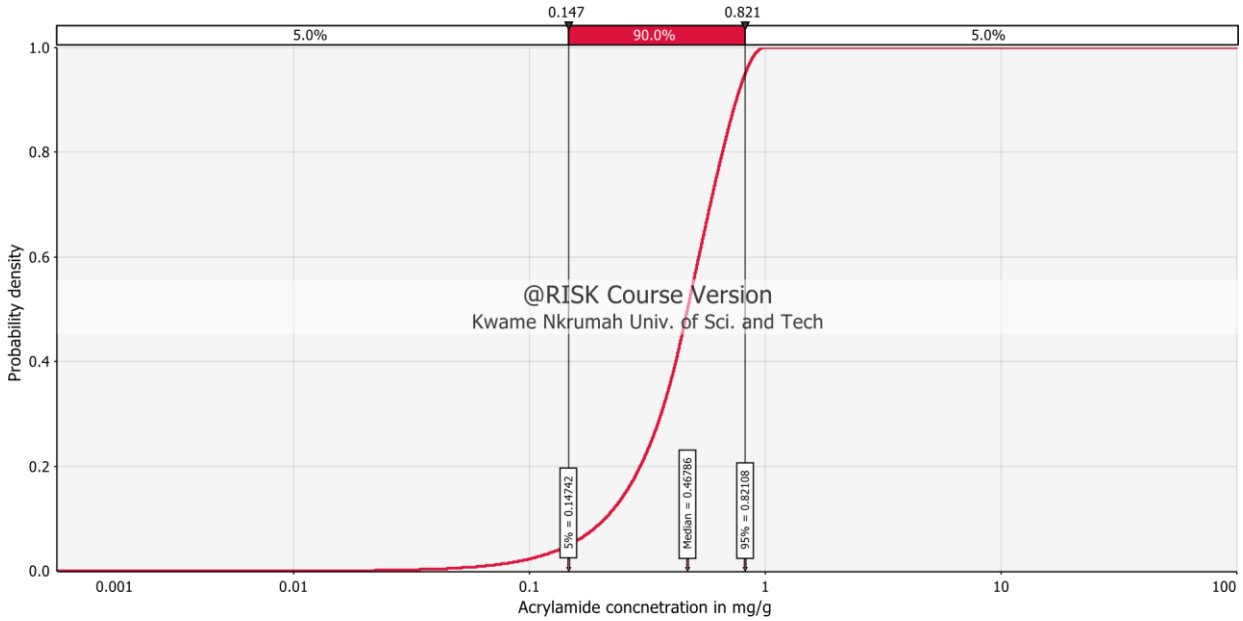


Figure 4.1: The probability density distribution of acrylamide concentration in buffloaf

4.3 Acrylamide exposure level in terms of CDI

Acrylamide exposure averaged across individuals' body weight over a lifetime of 70 years was estimated as realistic and stable for the present and future. The chronic daily intake (CDI), averaged over a life-time of seventy years for an assumed exposure duration of 365 days for each matrix was Monte Carlo simulated with ten thousand iterations. The assumed 70 year life-time is the standard value for carcinogenic CDI estimates (Gerba, 1999) of which carcinogens acrylamide is suspected to be. The assumed exposure period is realistic for a national staple. Also in risk estimations it is better to err on the side of overestimation rather than underestimation to avoid mass toxicity and to ensure consumer safety (Gerba, 1999), hence the justification of the exposure duration of a year assumed.

Acrylamide being a "probable human carcinogen" (IARC, 1994) does not have a reference dose. According to USFDA (2003), the average exposure in the US is 0.4 µg/kg/day (0.0004 mg/kgday) and worldwide range of 0.2-1.4 µg/kg/day (0.0002-0.0014 mg/kg-day) for 2005. Generally, all the mean values (0.12 to 0.16 mg/kg-day) and all the median values (0.0054 – 0.065 mg/kgday) of this current study exceeded this USFDA (2003) maximum (0.0014 mg/kg-day).

Table 4.5: The chronic daily intake (CDI), averaged over a life time of seventy years for an assumed exposure duration of 365 days for each matrix and iterated 10000 using Monte Carlo simulation

CDI(mg/kg-day)	Mean	Mode	5 % Percentile	50 % Percentile	95 % Percentile
Roasted plantain	0.1595	$7.1x10^{-5}$	0.0013	0.0645	0.6465
Roasted groundnut	0.0223	$1.4x10^{-5}$	0.0002	0.0086	0.0902
Smoked tuna (*Thunnus sp*)	0.1082	$9.3x10^{-5}$	0.0012	0.0436	0.4362
Tea bread	0.0488	$3.7x10^{-5}$	0.0006	0.0207	0.1941
Malt n Milk biscuit	0.0397	$2.7x10^{-5}$	0.0005	0.0166	0.1577
Chips	0.0180	$1.1x10^{-5}$	0.0002	0.0074	0.0720
Fried yam	0.0439	$2.4x10^{-5}$	0.0004	0.0172	0.1767
Fried plantain	0.0301	$2.8x10^{-5}$	0.0005	0.0141	0.1154

Fried *Trachurus japonicus*	0.0136	$7.6x10^{-6}$	0.0001	0.0057	0.0541
Buffloaf	0.0115	$1.1x10^{-5}$	0.0002	0.0054	0.0433
Roasted cocoyam	0.0615	$4.9x10^{-5}$	0.0008	0.0274	0.2361
Roasted yam	0.0495	$3.8x10^{-5}$	0.0007	0.0215	0.1943

The modal acrylamide exposure values obtained in this study ($1.00x10^{-5}$ to $7.06x10^{-5}$ mg/kg-day) as indicated by the CDI in Table 4.5 actually falls below the USFDA (2003) USA average of 0.0004 mg/kg-day and indeed the world minimum of 0.0002 mg/kg-day. By the median and mean CDI values the average exposure rate to acrylamide in the study population exceeded that of the USA and the world at large.

That is, it is expected that a single contact with a unit of acrylamide should induce carcinogenicity. It stands to reason therefore to compare exposure levels to critical findings elsewhere.

In contrast, however, the CDI values found in the study are well below values obtained from the Swedish populace (Svensson *et al*., 2003). The median acrylamide exposure for the Swedish people was 27 mg/kg-day. The 5^{th} percentile exposure was 9.1 mg/kg-day also far higher than values obtained in this study. Similarly the 95^{th} percentile was also reported as 62 mg/kg-day which is also much higher than that obtained in this study.

However, the modal CDI values indicated otherwise. That on the average worldwide the least exposed persons were more exposed than the most exposed person in the study area. This raises questions not of methodology, statistical methodology, but of the nature of the CDI. Should it call for pessimism or optimism as the means of safety? Such questions are best left to regulators to

decide given the circumstances of the policy formulation. We should err on the side of public safety and protect life.

The mean although appropriate to estimate the CDI, the CDI being a ratio data type, is however, susceptible to outliers, and skewness of the data distribution. As result, the mean may be unrepresentative (Chambliss and Schutt, 2015). The modal value on the other hand is more suitable for nominal data of which nominal data type CDI may not be classified as one. It furnishes the most probable CDI value. Its main disadvantage with regard to CDI estimation may be said to be that, if the frequency of the modal value is too low to be practical, it may not be typical of the dataset, of the population or in the case where the distribution is multimodal (Chambliss and Schutt, 2015). It is therefore possible that the modal CDI values obtained in this study are underestimates of the typical exposure. It is the advantage of the median therefore in the face of skewness and multimodal distribution of the dataset to typify the population.

For example, in the probability density distribution of buffloaf shown in Figure 4.1, it appeared clearly that the CDI distribution is skewed (skewness value of 3.6) to the right dragging along the mean with it. The median value however, stayed in position and may be a better representation of the CDI. This distribution is typical for the rest of the matrix. It is also evident from the graph that the mode may be a plausible typical since the distribution is unimodal.

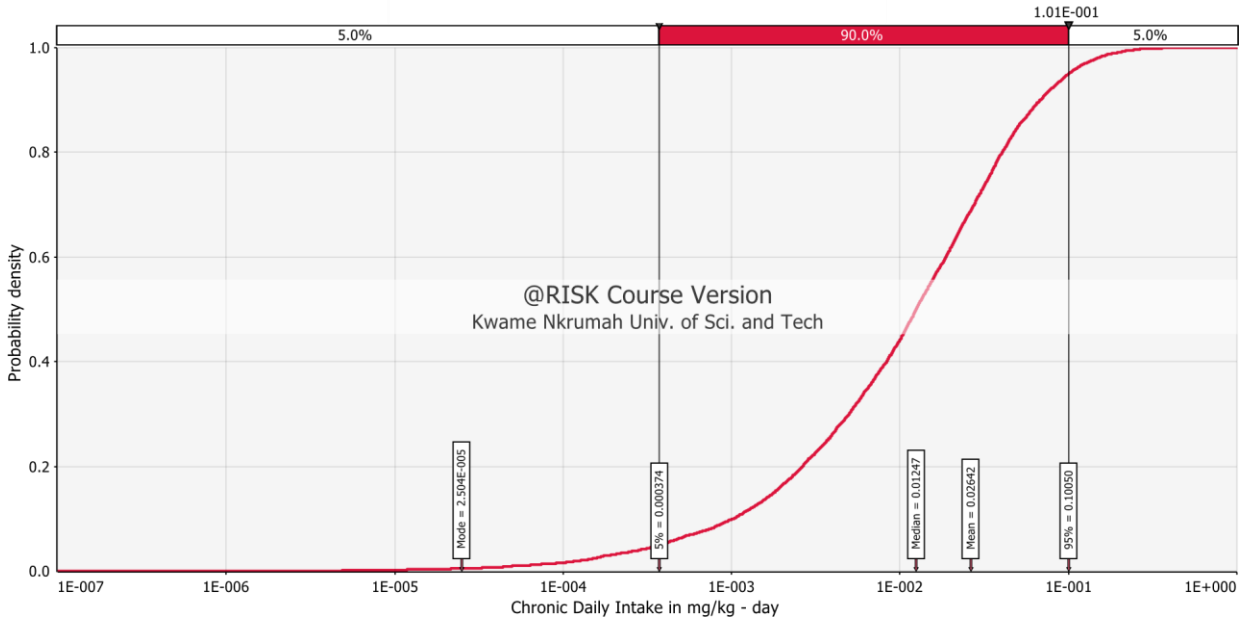


Figure 4.2: Probability density distribution of acrylamide exposure in buffloaf among the study population

Given the foregone argument and the fact that this is a risk assessment where overestimation furnishes safety (Gerba, 2009), it is the considered opinion of this study to use the median estimate of the CDI as the most realistic estimate of acrylamide exposure.

The 5 percentile group is made up of the least exposed group in the study population, they being the least vulnerable. They were found to be generally high compared to the US population and generally low compared to the Swedish population. For example, with respect to roasted plantain, the least exposed persons over a lifetime consumes about 0.0013 mg of acrylamide for each kilogram of body weight every day. This is higher than the average USA (0.0004 mg/kg-day) (USFDA, 2015) but lower than the median average Swede (27 mg/kg-day) and much lower than the 5th percentile group in Sweden (9.1 mg/kg-day) (Svensson *et al.*, 2003; USFDA, 2015).

The 95th percentile group is the most vulnerable group in the population and with respect to acrylamide have the highest exposure levels. The exposure ranges from 0.08 mg/kg-day for buffloaf to 4.66 mg/kg-day for roasted groundnut. These minimum and maximum 95th percentile mean acrylamide CDI from the study population were far below the 95th percentile typically reported for the Swedish population of 62 mg/kg-day.

4.4 Acrylamide carcinogenic and neurotoxic risk assessment for individual matrices

Here it is assumed that all things being equal should the population consume only a single matrix, to what extent would be the risk level? The results were revealing. The typical distribution of the simulated data for risk was skewed characteristically to the right. The probability density distribution had an estimated skewness of about 4. Consequently, the mean (0.006) has been dragged to the right far removed from the positional average, the median (0.003). This skewness was found for all the distribution of simulated risked results in this study. The median cancer risk (0.003) of 3 out of 1000 at the prevailing exposure rate is lower than the mean cancer risk of 6 out of 1000 persons. As a result, the median value may be a more realistic estimate of typical cancer risk in the study population. The modal value and the 5th percentile are very close as can be seen from Figure 4.3. The most probable risk of probable cancer for the current acrylamide exposure rate in the study area for buffloaf may be the modal value of 0.000005. It means that, it is likely that 5 out of 1,000,000 are at risk of probable cancer at the current rate of exposure. This level of risk is unacceptable with respect to the *de minimis* value, 1 out of 1,000,000. However, since it is safer to overestimate risk than to underestimate, the median risk level of acrylamide carcinogenicity of 3 out of a 1,000 may have to be critically looked at since this level of risk is unacceptable *de minimis.* A *de minimis* value is assumed to be a risk value equivalent within the range 10^{-4} to 10^{-6}, that is, a risk level from 1 out of a 10,000 to 1 out of 1,000,000 (Gerba, 1999).

Table 4.6: Mean, mode, 5th percentile, median and 95th percentile risk estimates for the study population for each matrix.

Cancer risk	Mean	Mode	5 % Percentile	50 % Percentile	95 % Percentile
Roasted plantain	0.08	$3.0x10^{-05}$	0.0007	0.032	0.30
Roasted groundnut	0.01	$6.6\ x10^{-06}$	0.0001	0.004	0.04
Smoked tuna (*Thunnus sp*)	0.05	$3.3\ x10^{-05}$	0.0006	0.022	0.22
Tea bread	0.02	$2.4\ x10^{-05}$	0.0003	0.010	0.10
Malt n Milk biscuit	0.02	$1.6\ x10^{-05}$	0.0003	0.008	0.08
Chips	0.01	$6.9\ x10^{-06}$	0.0001	0.004	0.04
Fried yam	0.02	$1.2\ x10^{-05}$	0.0002	0.008	0.09
Fried plantain	0.01	$1.4\ x10^{-05}$	0.0002	0.007	0.05
Fried *Trachurus japonicus*	0.01	$4.6\ x10^{-06}$	0.0001	0.003	0.03
Buffloaf, Cancer Risk	0.01	$5.4\ x10^{-06}$	0.0001	0.003	0.02
Roasted cocoyam	0.03	$2.3\ x10^{-05}$	0.0004	0.013	0.12
Roasted yam	0.03	$1.9\ x10^{-05}$	0.0003	0.011	0.10
Risk of degenerative nerve disorders					
Roasted plantain	0.18	$-9.3\ x10^{-04}$	0.0006	0.073	0.71
Roasted groundnut	0.02	$-9.8\ x10^{-04}$	-0.0007	0.009	0.10
Smoked tuna (*Thunnus sp*)	0.12	$-9.2\ x10^{-04}$	0.0004	0.050	0.50
Tea bread	0.06	$-9.4\ x10^{-04}$	-0.0003	0.023	0.22
Malt n Milk biscuit	0.05	$-9.6\ x10^{-04}$	-0.0004	0.018	0.19
Chips	0.02	$-9.8\ x10^{-04}$	-0.0007	0.008	0.08
Fried yam	0.05	$-9.7\ x10^{-04}$	-0.0004	0.019	0.21
Fried plantain	0.03	$-9.7\ x10^{-04}$	-0.0004	0.016	0.13
Fried *Trachurus japonicus*	0.01	$-9.9\ x10^{-04}$	-0.0008	0.006	0.06
Buffloaf, Cancer Risk	0.01	$-9.9\ x10^{-04}$	-0.0008	0.005	0.05
Roasted cocoyam	0.07	$-9.5\ x10^{-04}$	-0.0001	0.030	0.27
Roasted yam	0.06	$-9.6\ x10^{-04}$	-0.0002	0.024	0.23

For the purpose of this study the upper bound of this *de minimis* value, that is a risk level of 1 out of a 1,000,000, may have to be strictly used for probable cancer risk. This is because cancer is a non-threshold response to chemical carcinogens, and that zero acrylamide exposure is desirable, since it is assumed that a single exposure to a molecule of acrylamide may probably cause cancer.

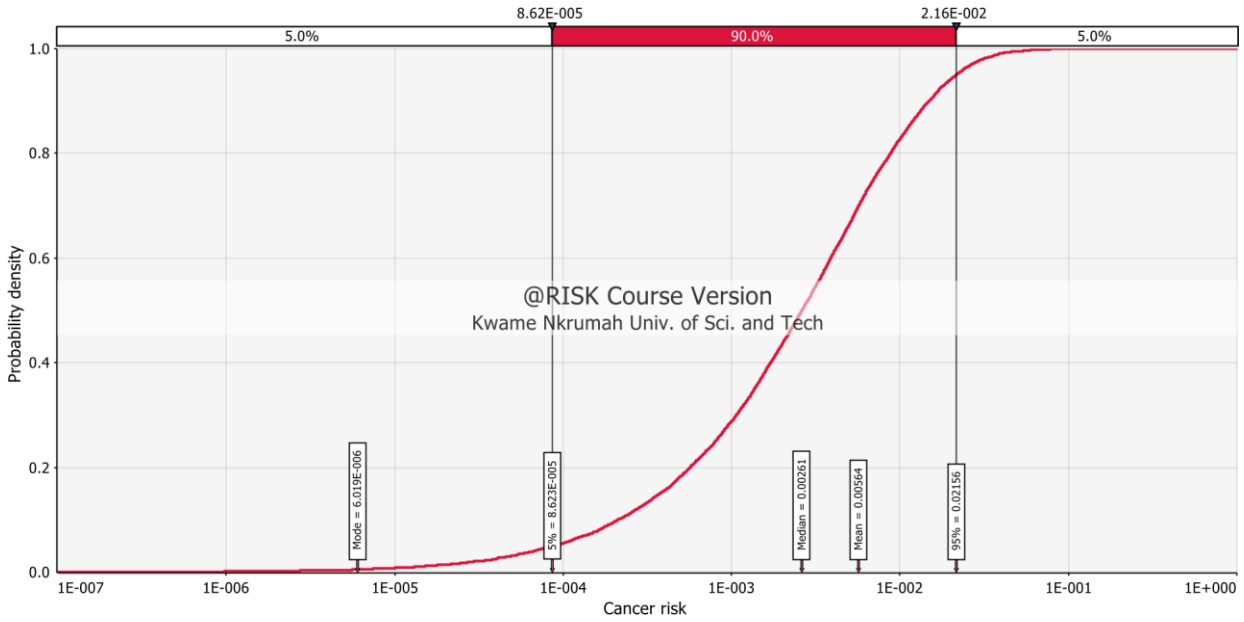


Figure 4.3: Distribution of cancer risk in buffloaf

Although this *de minimis* level of risk is considered trivial, other perceptional factors may render them significant. As such, even a supposedly trivial level of risk as this, may have to be interpreted within the context of other factors of risk that derive from such disciplines as sociology, psychology and economics. A perceptional analysis of the risk of acrylamide is beyond the scope of this work.

In this work, the expression *de minimis* means "with respect to the value, 10^{-6}" for probable cancer and "with respect to the value, 10^{-4}-10^{-6}" for neurotoxicity.

For the risk of degenerative nerve disorders, the median value was found to be 5 out of 1,000 for buffloaf. The values for most likely or modal level of risk were negative as was the 5^{th} percentile level: -0.001 for the former and -0.01 for the latter. This means that at their respective levels of consumption rate, they are not at risk at all to any acrylamide toxicity through the consumption of buffloaf. This absence or existence of negative risk is explained in the empirical model by their

CDI being less than the RfD. The 5th percentile CDI was 0.0002 mg/kg-day and the modal CDI was 1.1x10^{-5} mg/kg-day which are far less than the EPA validated acrylamide RfD of 0.002 mg/kgday (IRIS, 2010) for degenerative nerve disorders.

4.4.1 Roasted plantain

The risk of cancer for roasted plantain was found to be 8 out of 100 using the mean but 3 out of 100 using the median, the median value giving a lower level of risk for reasons of skewness discussed earlier. For yet more reasons discussed previously, we use the median value as typical of the population, this value being unacceptable *de minimis*. The probable risk value was 2 out of a 100,000 which is acceptable *de minimis.* The most vulnerable population at the prevailing acrylamide exposure have risk value 0.30, which means that 3 out of 10 are at risk of cancer. The least vulnerable members, the 5th percentile group, have a risk level of 8 out of 100 which is also unacceptable *de- minimis.*

The risk of degenerative nerve disorder for plantain using the median is high at seven out of hundred and unacceptable *de minimis* as was the 5th (6 out of 10,000) and 95th percentile (7 out of 10). The most probable risk was however negative, meaning they are not at risk at all. According to the fit of the empirical model, the modal CDI for roasted plantain was 7.06x10^{-05} mg/kg-day which is far less than the 0.002 mg/kg-day RfD value.

4.4.2 Roasted groundnut

The most probable risk which is the modal value was found to be 7 out of 1,000,000 which is acceptable *de minimis*. However, the median value was found to be 4 out of 100 which is unacceptable *de minimis*. The 5th percentile members of the population being the least vulnerable are 0.0001 at risk, which means that one out of 10,000 members are at risk of cancer due to acrylamide by the oral route in roasted groundnut. The most vulnerable group however have a risk

level of 0.01, that is, 1 out of 100 members of the population are at risk of cancer, which is also *de minimis* unacceptable.

The median value was found to be 9 out of 1,000 for degenerative nerve diseases, which is unacceptable *de minimis*. The 95th percentile group was 7 out of 10 degenerative nerve disease risk which was unacceptable *de minimis.* On the other hand, the 5th percentile have a risk of degenerative nerve disorder of which is negative and so consume acrylamide at a CDI level (0.0002 mg/kg-day) that is less than the RfD of 0.002 mg/kg-day. So was the modal risk at a modal CDI of 1.469x10^{-05} mg/kg-day.

4.4.3 Smoked tuna

The median risk was found to be 2 out of 100 members of the population who are at risk of cancer due to current exposure rate of acrylamide in smoked tuna, *Thunnus sp,* this value being *de minimis* unacceptable. The most probable value of risk for smoked tuna was 3 out of a 100,000 which is low but unacceptable *de-minimis*. It was estimated that 6 out of 10,000 for the 5th percentile group and 2 out of 10 for the 95th percentile group were found to be at risk of probable cancer, both being unacceptable *de minimis.*

The most probable risk of degenerative nerve disorder was found to be negative for the consumption of smoked tuna at a modal CDI level of 9.32 x10^{-05} mg/kg-day that is less than the acrylamide RfD. The corresponding risk for the 5th percentile, the median and the 95th percentile were four out of ten thousand, five out of hundred and five out of ten respectively. These values are clearly below the *de minimis* range and are as such unacceptable.

4.4.4 Tea bread

For the current acrylamide exposure through tea bread consumption, the cancer risk level is one out of hundred according to the median. The 5th percentile level of cancer risk was found to be one

out of hundred and the 95th percentile about one out of ten. Both are unacceptable *de minimis*. The most probable risk level was estimated to be 2 out of 100,000, which is very low but still unacceptable *de minimis.*

With respect to degenerative nerve disease, the risk for the consumption of acrylamide in tea bread was found to be negative for the mode and 5th percentile values because their respective CDIs of 3.68x10^{-5} mg/kg-day and 0.0006 mg/kg-day were less than the 0.002mg/kg-day RfD. There is no probable risk for the population as a whole and the 5th percentile group. However, the 2 out of 10 members of the most vulnerable members of the population are at risk of degenerative nerve disease.

4.4.5 Biscuit

The median value was found to be 8 out of a 1,000 probable cancer risk through biscuit. This value is unacceptable *de minimis*. The most vulnerable group, 95th percentile, 8 out of 100 risk of probable cancer being *de minimis* unacceptable as the 5th percentile group's probable cancer risk of 3 out of 10,000. The most likely risk of probable cancer was found to be 2 out of 100,000 which is low but unacceptable *de minimis*.

Neurotoxicity, the most likely risk level or the modal risk level and the 5th percentile risk levels were negative at a respective CDI level of 2.72x10^{-05} mg/kg-day and 0.0005 mg/kg-day, well below 0.002 mg/kg-day acrylamide RfD. On the other hand, the median and 95th percentile groups had neurotoxic risk levels of 2 out of 100 and 2 out of 10 respectively, which is high and unacceptable *de minimis.*

4.4.6 Chips

The median probable cancer risk was found as four out of a thousand this is *de minimis* unacceptable. The 95th percentile (4 out of 100) was found to be *de minimis* unacceptable as was the 5th percentile (1 out of 10,000) risk. The most probable risk was found to be 7 out of 1,000,000 which is unacceptable *de minimis.*

The neurotoxic risk of acrylamide was found to be negative for chips according to the median and the 5th percentile risk values at $1.14x10^{-05}$ mg/kg-day and 0.0002 mg/kg-day CDI respectively, which were less than the 0.002 mg/kg-day. The 95th percentile and median risk of neurotoxicity were however unacceptably high compared to the *de minimis* and are therefore unacceptable.

4.4.7 Fried yam

The modal risk for only fried yam acrylamide exposure was found to be 1 out of 100,000 which is well within the *de minimis* acceptability range. The median (8 out of 1000), 5th percentile (2 out of 10,000) and 95th percentile probable cancer risk were however unacceptable *de minimis*.

The neurotoxicity of acrylamide risk for fried yam was estimated and found to be negative according to the mode and 5th percentile. It means at the modal level of exposure there is no risk of acrylamide just as at the 5th percentile level. The risk of neurotoxicity however, was found to be high for the median (2 out of 100) and the 95th percentile (2 out of 10) and unacceptable *de minimis*.

4.4.8 Fried plantain

The median probable cancer risk was found as 7 out of 1000; this is *de minimis* unacceptable. The 95th percentile (9 out of 1000) was found to be *de minimis* unacceptable as was the 5th percentile (2 out of 10,000) risk. The most probable risk was found to be 7 out of 1,000,000 which is acceptable *de minimis* for acrylamide fried plantain exposure prevailing. The most probable level

of risk for the population was found to be negative and so typically the study population may be said not to be at risk for acrylamide neurotoxicity. This also pertains at the 5th percentile group. However, at a risk level of 2 out of 100 for the median, positional average indicates otherwise on the basis of *de minimis*. Furthermore, the most vulnerable group have a neurotoxic risk of 1 out of 10 which is very high and unacceptable *de minimis*.

4.4.9 Fried *Trachurus japonicus*

For the current acrylamide exposure through fried *Trachurus japonicus* consumption, the probable cancer risk level is 2 out of a 1000 according to the median. The 5th percentile level of probable cancer risk was found to be 7 out of 100,000 and the 95th percentile about 3 out of 100. Both are unacceptable *de minimis*. The most probable risk level was estimated to be 5 out of 1,000,000 which very low and acceptable *de minimis.*

At a neurotoxic risk level of 6 out of 1000 and 6 out of 100 as the median and 95th percentile risks are both unacceptable *de minimis*. However, the modal risk and 5th percentile equivalents are negative and so the population may be said not to be at risk.

4.4.10 Roasted cocoyam

The median risk was found to be 1 out of 100 members of the population being at risk of probable cancer due to current exposure rate of acrylamide in roasted cocoyam, this value being *de minimis* unacceptable. The most probable value of risk for buffloaf was 2 out of 100,000 which is unacceptable *de minimis*. Four, 4, out of 10,000 for the 5th percentile group and 1 out of 10 for the 95th percentile group were found, both being unacceptable *de minimis.* The most likely risk for neurotoxicity was found to be negative and so it is likely that the population is not at risk, so are the 5th percentile group. On the contrary, the median neurotoxic risk was 3 out of 100 and unacceptable according to *de minimis*, so was the 95th percentile at 2 out of 10.

4.4.11 Roasted yam

Consumption of roasted yam only predisposes the population to a probable cancer risk level of 1 out of 100 by median, 1 out of 10 at the 95th percentile level and 3 out of 10,000 at the 5th percentile level all of which are unacceptable *de minimis.* The most probable risk level typical of the population was estimated as 2 out of 100,000 risk level which is low but unacceptable *de minimis.*

The risk of acrylamide neurotoxicity via roasted yam is unacceptable at the 95th percentile (2 out of 100) and at the median (2 out of 10) relative to the *de minimis*. However there is no risk for the 5th percentile group since the value is negative. The most probable risk is also negative and as such it is most likely that the population is not at risk of acrylamide neurotoxicity.

4.5.1 Overall acrylamide estimates

A presentation of the risk parameters and the risk status in a scenario where the population is analyzed with respect to all the matrices. This aggregate analysis gives a realistic overall picture since members of the population consume different combination of the matrices at different times for different individuals.

4.5.2 Aggregate acrylamide concentration

From Table 4.7, the combined acrylamide concentration of all the matrices: roasted plantain, roasted groundnut, smoked tuna (*Thunnus sp.*), tea bread, biscuit, chips, fried yam, fried plantain, fried *Trachurus japonicas,* buffloaf, roasted cocoyam and roasted yam- was found to be 1.75 mg/g as mean, 0.27 mg/g as mode and 1.56 mg/g as median.

Table 4.7: Overall parameter estimates of acrylamide related matrices in the study.

Parameter	Mean	Mode	5 % Percentile	50 % Percentile	95 % Percentile
Acrylamide concentration/mg/g	1.7526	0.2682	0.2556	1.5568	3.9078
No. of times consumed/per day	0.43	0.00	0.03	0.38	1.00
Mass of medium/g	1062.6000	1817.0530	106.2021	1062.4970	2018.7990
Ingestion rate/g/day	456.5303	0.8740	13.0631	302.0440	1407.1700
Weight of respondent/kg	60.11	53.84	18.47	58.73	104.94
Cancer CDI (mg/kg-day)	0.2669	0.0002	0.0030	0.0998	0.9705
Cancer Risk	0.1	8.3E-05	0.001	0.05	0.5
Non-cancer CDI(mg/kg-day)	0.6221	0.0004	0.0069	0.2273	2.6430
Non-cancer Risk	0.3	-0.0008	0.002	0.1	1

According to Figure 4.4, the acrylamide concentration distribution is skewed to the right (a skewness value of about 1). It is appropriate to rely on the median, 1.56 mg/g, as the typical value of acrylamide in all the matrices analyzed in the study and not the mean. This is because the arithmetic mean would be distorted to the right but this distortion would not affect the median. However the most probable acrylamide concentration for all the matrices may be said to be the mode, 0.27 mg/g. This value is higher than the EFSA (2012) indicated value of 0.001 mg/g and therefore calls for further assessment. Attribution may be assigned to longer cooking hours, overcooking and higher cooking temperature.

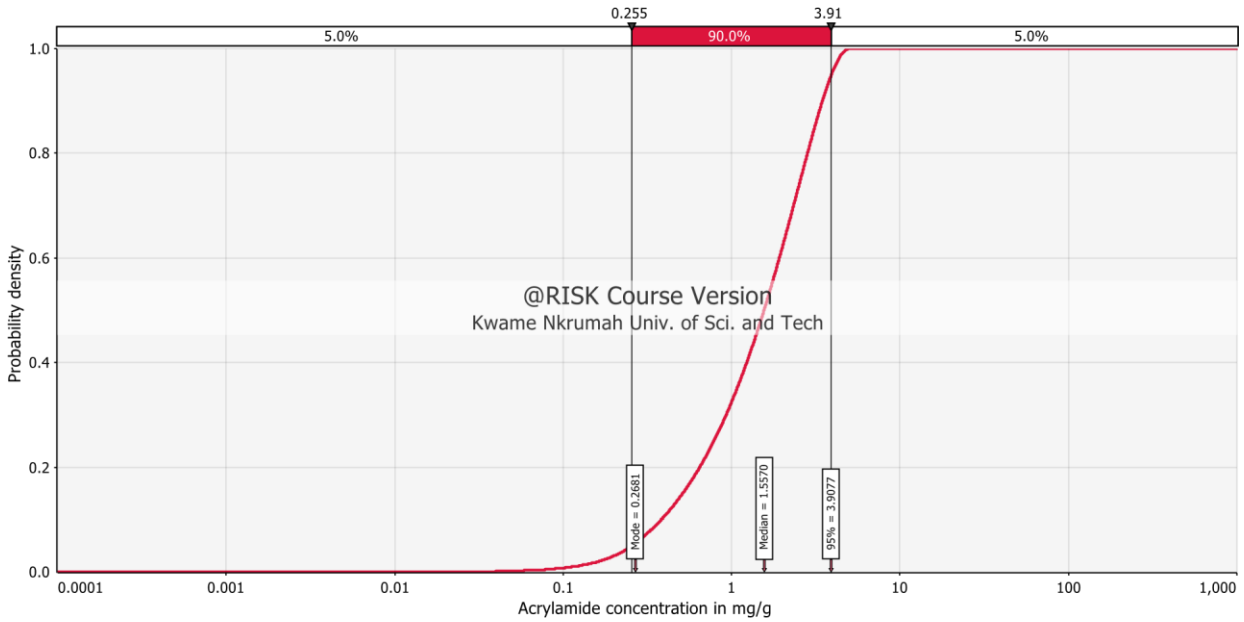


Figure 4.4: Cumulative probability density distribution of acrylamide concentration in the aggregate matrix of the study

The 5th percentile value was found to be 0.26 mg/g. They are made up of 5 % of the sampled matrices with the least acrylamide concentration. This also is higher than the EFSA (2012) indicative value of 0.001 mg/g and calls for further assessment. That the 5th percentile value exceeds the indicative value, may imply that most of the food matrices in the study population are either heated at high temperatures, heated for long periods and or are overcooked. As expected, the 95th percentile value of the acrylamide concentration in the food matrices was way higher than the EFSA (2012) indicative value of 0.001 mg/g and consumers at this level of concentration are expected to have a greater exposure to acrylamide and therefore a greater risk of acrylamide toxicity.

Since there is a skewness of about 1 in the distribution in the number of consumption in a day, the mean is correspondingly distorted to the right. So we rely on the median of 0.377 times a day as a positional average that is insensitive to skew to be the typical consumption rate of the matrices in

the study. This translates to less than three times a week. The 5th percentile value is 0.033 which translate to less than once every month.

4.5.3 Overall consumption rate of acrylamide

A consumption rate of all the matrices was found to be 0.428, 0.003 and 0.377 times a day by mean, mode and median in that order.

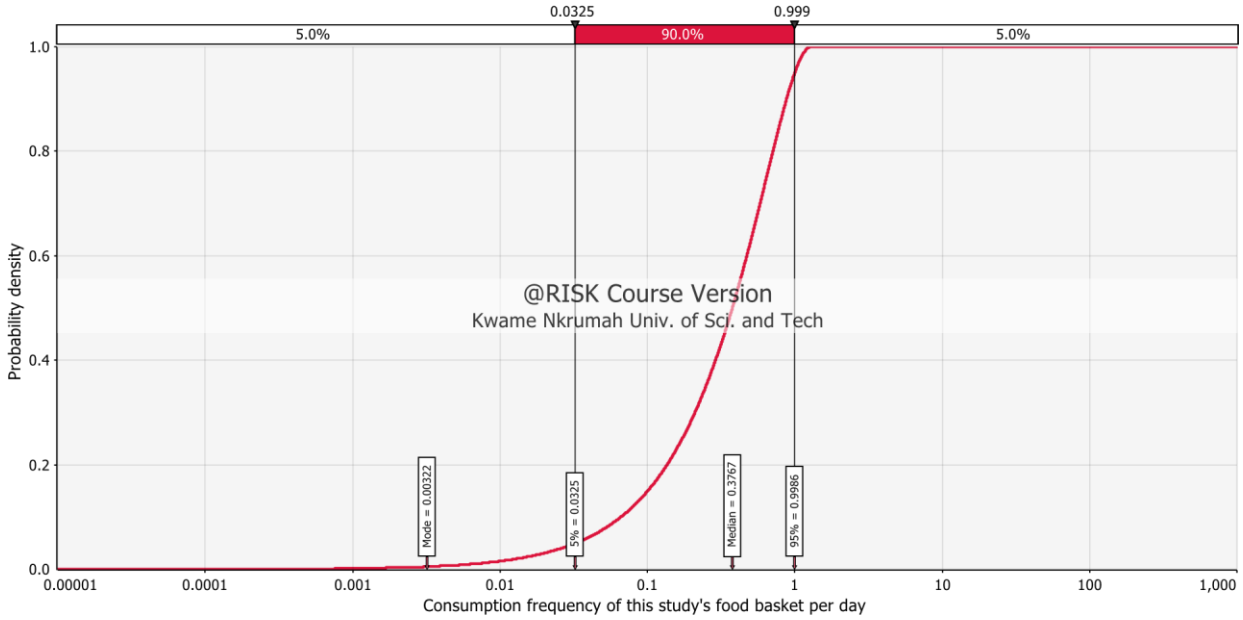


Figure 4.5: Cumulative probability density frequency of consumption for the aggregate matrix in a day

The consumption rate for the 95th percentile was found to be 0.999, that is, about once a day. This high level of consumption, given a corresponding high value of acrylamide concentration, may predispose people to high risk of acrylamide toxicity.

4.5.4 Matrix ingestion rate

The aggregate estimated ingestion rate of the matrices in the study was 456.53 g/day, 0.87 g/day and 302.04 g/day as found in Figure 4.6.

The distribution of the ingestion rate output was skewed to the right with a skewness value of 1.4 and so we would rely on the median as the typical value. That means, typically, 302.04 g was consumed a day by members of the population. The 5th percentile value was 13.06 g/day and the 95th percentile value was 1411.52 g/day.

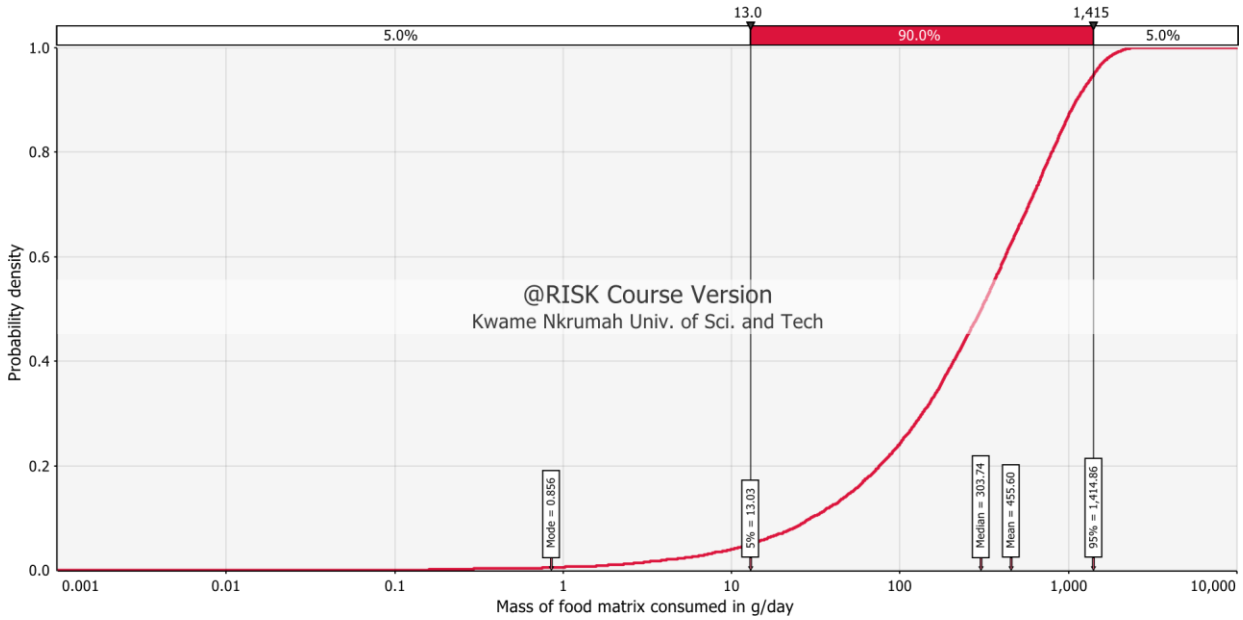


Figure 4.6: Overall ingestion rate distribution.

The alter ingestion rate coupled with a high expose predisposes to risk of acrylamide toxicity. The modal ingestion rate was relatively low, 0.87 g/day.

4.5.5 Weight of respondents

The mean, mode and median were 60.11kg, 54.47 kg and 58.73 kg in that order. The distribution (Figure 4.7) is not quite symmetrical, having a skewness (0.14), howbeit slight. The mean and median are as such close but different. The 5th and 95th percentiles were 18.47 kg and 104 kg. The former may be children and the later adults or may be obese.

4.5.6 Overall Chronic Daily Intake estimate for cancer as a measure of acrylamide exposure

The mean, modal and median values were estimated from the empirical data simulated as 0.27 mg/kg-day, 0.002 mg/kg-day and 0.10 mg/kg-day. From figure 4.8, the distribution of the CDI is highly skewed to the right, with a skewness value of 22, as such the mean is accordingly distorted.

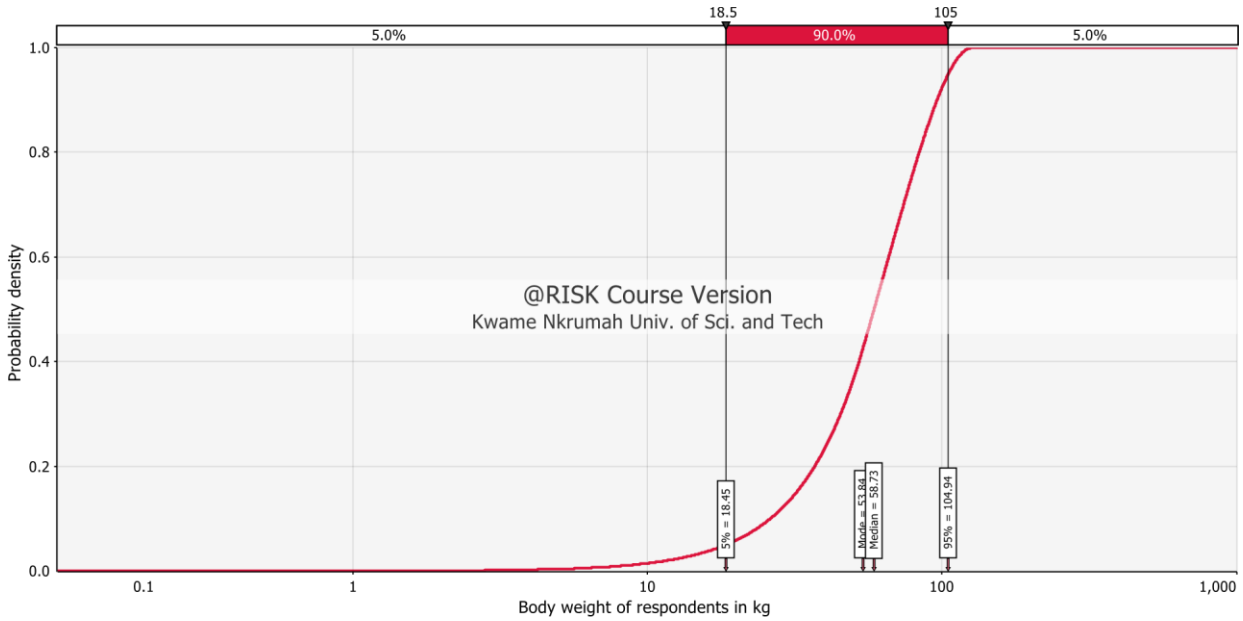


Figure 4.7: A distribution of body weight of respondents.

The study should therefore rely on the median as a typical CDI value of the population. This typical 0.10 mg/kg-day CDI for the study population is higher than the typical exposure value of 0.0004 mg/kg-day (USFDA, 2015) for the US population but lower than the typical of 27 mg/kg-day for the Swedish population (Svensson *et al*., 2003). The 5th percentile and 95th percentile CDIs were 0.0029 mg/kg-day (much lower than the Swedish 5th percentile of 9.1 mg/kg-day) and 0.97 mg/kgday respectively. The latter being much lower than the 95th percentile value (62 mg/kg-day) of the Swedish population (Svensson *et al*., 2003).

4.5.7 Overall Non-cancer CDI

From chapter 3, the CDI of cancer was estimated using 70 life time average and that of negative nerve disease was estimate using 30 years according to the conventions of risk analysis (Gerba, 1999). The exposure values there for non-cancer are not averaged over a lifetime but over a period of thirty years. The study therefore expects the CDI of non-cancer to be naturally higher than corresponding values for cancer, *ceteris paribus*.

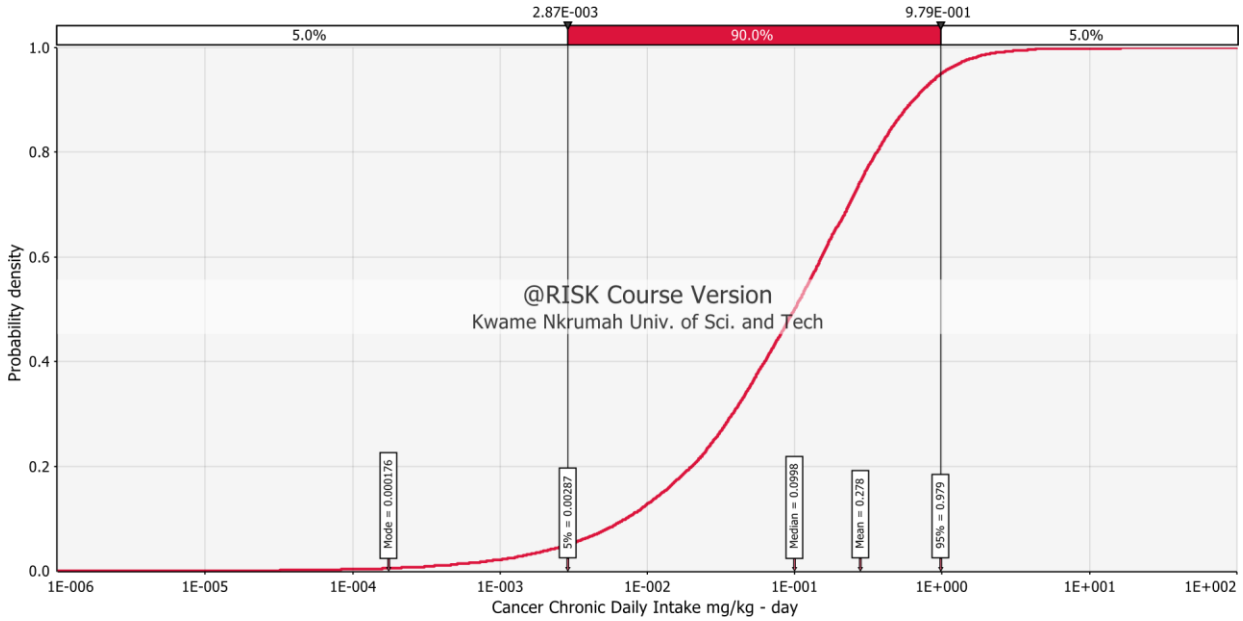


Figure 4.8: Cumulative probability density distribution of overall cancer CDI

Accordingly, we find the mean, mode and median 0.62 mg/kg-day, 0.00039 mg/kg-day and 0.23 mg/kg-day respectively greater than corresponding values for cancer CDIs (0.27 mg/kg-day, 0.002 mg/kg-day and 0.100 mg/kg-day respectively) except the modal value which was found to be less. As was the case for the CDI distribution for cancer (Figure 4.8), the CDI distribution (Figure 4.9) for non-cancer is skewed to the right with a skewness value also of 22, and so we use the median as the typical value for CDI. The fifth and ninety-fifth percentiles are 0.0069 mg/kg-day and 2.64 mg/kg-day. Again the typical CDI of non-cancer acrylamide toxicity was greater than the typical US values but less than corresponding values of Sweden (Svensson *et al.*, 2003; USFDA, 2015).

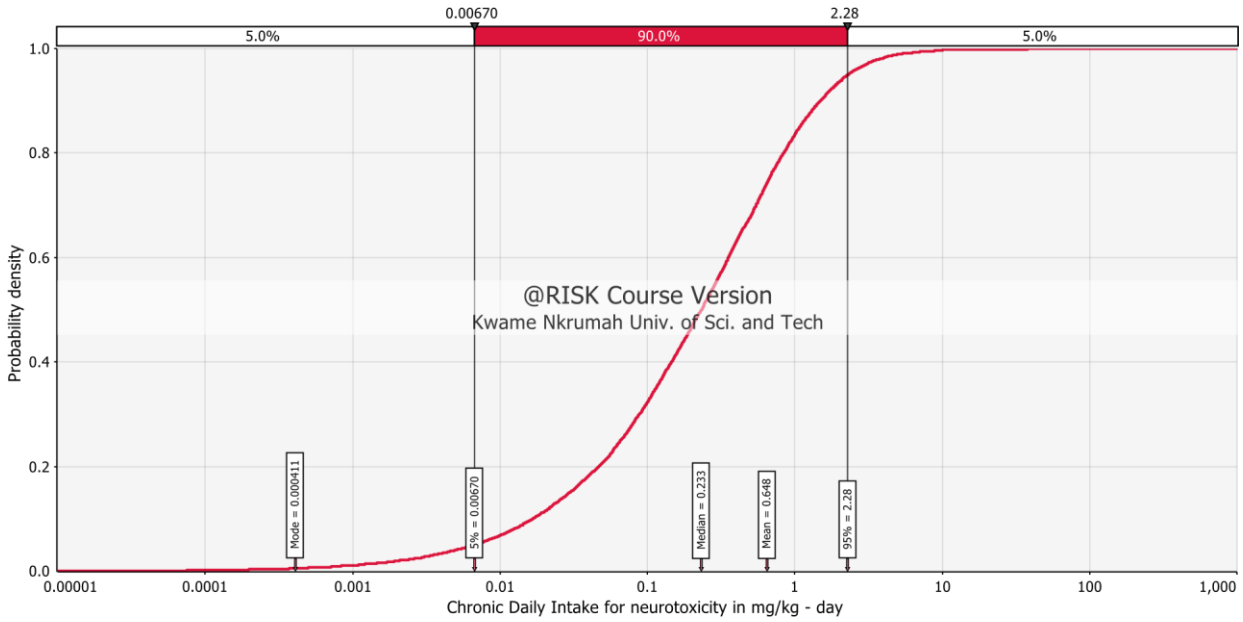


Figure 4.9: Cumulative probability density distribution of non-cancer CDIs

4.5.8 Overall acrylamide probable carcinogenicity risk by the oral route

From figure 4.10, it could be seen that the mean, modal and median cancer risk were estimated as 0.1, 0.00008 and 0.05 respectively. The study should use the median risk because the risk distribution was skewed to the right (skewness value of 22). So the typical value for probable cancer risk for the prevailing exposure rate was found to be 5 out of 100. That means 5 out of 100 members in the population are at risk to probable cancer due to acrylamide. This value is unacceptable *de minimis*. The most probable risk of probable cancer estimate, which is the mode, is 8 out of 100,000 which is also unacceptable *de minimis*. The fifth percentile risk was estimated at 1 out of 1000 still unacceptable *de minimis*. Thus, the least vulnerable members of the population are at risk of probable cancer due to acrylamide. The ninety-fifth percentile made up of the most vulnerable group are at risk to probable cancer *de minimis* due to acrylamide at a level of 5 out of 10. This is serious because this estimate is only for the oral route in foods. Other routes such as the

nasal route and dermal route should they be included, may escalate the risk of probable acrylamide carcinogenicity.

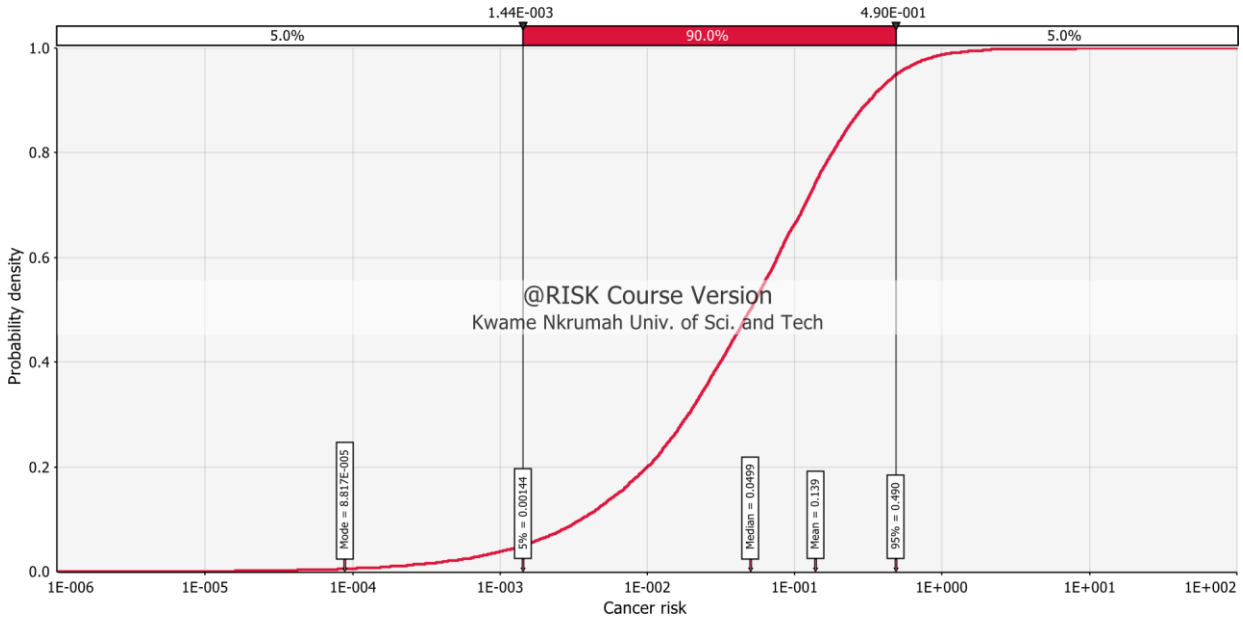


Figure 4.10: Cumulative probability density distribution of simulated cancer risk due to acrylamide consumption.

4.5.10 Overall neurotoxicity of acrylamide by the oral route

The neurotoxicity of acrylamide was found to be 0.3, -0.0008 and 0.1 for the mean, mode and median respectively. From the distribution of risk in Figure 4.11, it could be realized that there is a skew of 14. As such, the study should use the median as a typical risk of neurotoxicity due to acrylamide which was found to be 1 out of 10, this risk level being higher than the *de minimis* is unacceptable. The modal neurotoxic risk is negative. This means that the modal CDI, overall modal CDI, for neurotoxicity was less than the modal RfD. As a result, it can be conclusively stated that with respect to the mode, there is no risk of acrylamide neurotoxicity currently for the consumption of the matrices in this study. The fifth percentile risk in this category was also found to be 1 out of 100 which is also unacceptable *de minimis*. The ninety-fifth percentile was also estimated at 5 out of 10 which is very high and unacceptable *de minimis*.

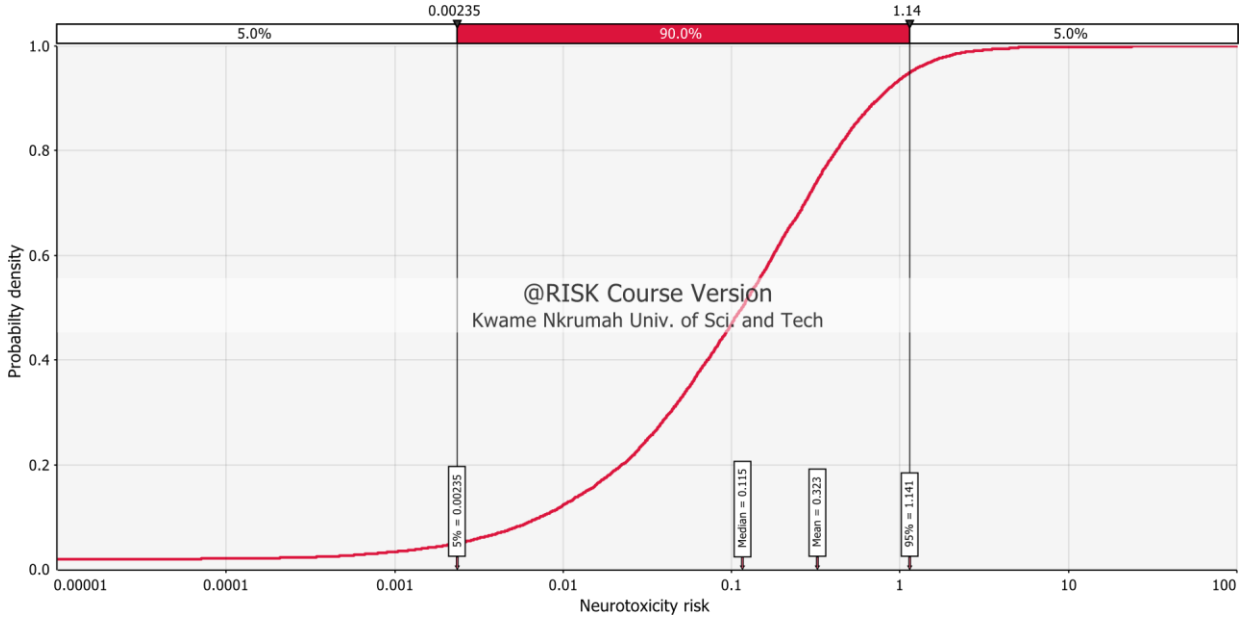


Figure 4.11: Cumulative probability density distribution of neurotoxicity risk of acrylamide through the oral route by food.

4.5.11 Sensitivity analysis of the risk with respect to the model inputs

With respect to the tornado graph in Figure 4.12, a standard deviation decrease in the weight of the respondent in kilogram (25.90 kg) would increase the baseline risk, which is the mean risk value, of one out of ten to four out of ten. A standard deviation increase in the weight should decrease the risk of cancer from 1 out of 10 (baseline) to 5 out of 100. Similarly, a standard deviation increase (0.30 per day, about 3 times in 10 days) in the number of times of consumption should increase the risk from the baseline of one out of ten to 3 out of 10 whereas a corresponding decrease should decrease the baseline to 1 out of 100. It should take a standard deviation increase (1.15 mg/g) in the concentration of acrylamide in the matrix to increase the baseline risk to 3 out of 10 and a decrease of the same to reduce the baseline to 2 out of 100.

Decreasing the mass of medium consumed by one standard deviation which is 613.49g, should decrease risk of cancer to 1 out of 100 from the baseline and increasing the mass of medium consumed by one standard deviation should increase risk to 3 out of 10 from the baseline.

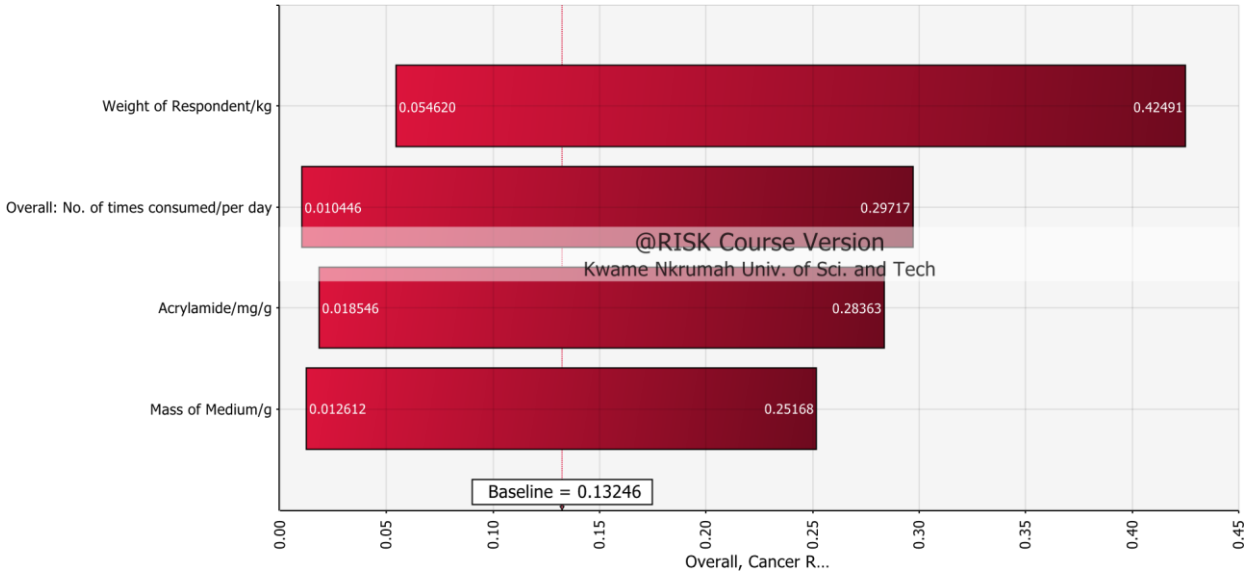


Figure 4.12: Respondent weight, consumption frequency, acrylamide concentration, and mass of medium ranked by effect on overall cancer risk.

In the light of the forgone analysis, it could be deduced that cancer risk is most sensitive to the weight of consumers, followed by frequency of consumption, then acrylamide concentration in that order. Mass of medium is least sensitive to cancer risk. It means that in the empirical model for this study, a relatively small change in weight of consumers affects the mean level of risk most. As a result, risk mitigation should target managing weight and frequency of consumption in the short to medium term and concentration and mass of medium in the long term.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The typical concentration of acrylamide in the matrices were found as buffloaf 0.48 mg/g, fried plantain 1.30 mg/g, tea bread 1.31 mg/g, chips 1.34 mg/g, fried fish 1.58 mg/g, fried yam 1.65

mg/g, roasted cocoyam 1.70 mg/g, roasted yam 1.70 mg, smoke tuna 1.79 mg/g, biscuit 1.95 mg/g, roasted groundnut 2.08 mg/g and roasted plantain 2.34 mg/g. These levels we found to be higher than the indicative value of EFSA (2012), 0.001 mg/g but generally less than the maximum for Sweden, 2.3 mg/g.

The mean acrylamide concentration in the matrices were estimated across processing methods as roasted 1.88 mg/g, grilled 1.79 mg/g, baked 1.60 mg/g, and fried 1.42 mg/g in order of decreasing concentration. A Tukey's multiple comparisons test on these means at 95% confidence interval showed that the differences between them were all significant.

Using a t test at 95% confidence interval, there was a significant difference between the means of acrylamide in the roasted and fried plantain, with that of the roasted (1.30 mg/g) being higher than that of the fried (2.34 mg/g). Similarly, there also existed a significant difference between acrylamide concentration means of fried yam and roasted yam, with that of the roasted yam (1.70 mg/g) being higher than that of the fried yam (1.65 mg/g). The typical exposure values for acrylamide were found to range from 0.0054 mg/kg-day to 0.065 mg/kg-day for the individual matrices and they all exceeded USFDA (2003) maximum (0.0014 mg/kg-day) but fell far below exposure for the Swedish people, 27 mg/kg-day.

For the individual matrices, the cancer risk ranged from 3 out of 100 to 3 out of 1000 using the median. This is unacceptable *de minimis*. However, using the mode, individual risk for cancer due to acrylamide were generally low and close to the *de minimis* value. There is higher cancer risk for individual matrices at the 95^{th} percentile than for the 50^{th} percentile.

The modal risk for neurotoxicity was negative for all individual matrices, and as such, by the most probable typical value, are not at risk to neurotoxicity. Using the median risk however, they were all found to be at risk compared to the *de minimis* value, and that the 95th percentile risk were higher than the median risk to neurotoxicity.

The typical value of acrylamide in all the matrices analyzed in the study was 1.56 mg/g. This value is higher than the EFSA (2012) indicated value, 0.001 mg/g, and therefore calls for further assessment. The typical consumption frequency of all the combined matrices was found to be 0.377. This translates to less than three times a week. The matrix ingestion rate was estimated to be 302.04 g/day. The typical weight of the respondent was 58.73 kg.

The study found the typical exposure rate to acrylamide averaged over a life time of 70 years and over individuals to be 0.001 mg/kg-day. This typical 0.100 mg/kg-day CDI for the study population is higher than the typical exposure value of 0.0004 mg/kg-day for the US population but lower than the typical of 27 mg/kg-day for the Swedish population.

The most probable risk estimate for probable cancer due to acrylamide, which is the mode, was found by the study to be 8 out of 100,000 which is also unacceptable *de minimis*. The typical value for cancer risk for the prevailing exposure rate was found to be 5 out of 100. That means 5 out of 100 members in the population are at risk to cancer due to acrylamide. This value is unacceptable *de minimis*.

The median as a typical risk of neurotoxicity due to acrylamide was found to be 1 out of 10, this risk level is higher than the *de minimis* and is unacceptable. The most probable risk, the mode was

found to be negative. By the latter criteria, the study population is not at risk. A sensitivity analysis indicated that cancer risk is most sensitive to the weight of consumers. It is then followed by the frequency of consumption and acrylamide concentration. The mass of media is the least sensitive to probable cancer risk.

5.2 Recommendations

Since the level of acrylamide in the matrices overall was higher than the indicative value set by EFSA (2012), the study recommends that further investigation should be done on them to identify the factors that account for their high levels and how they could be managed to reduce the acrylamide levels to below 0.001 mg/g. In doing so, the processing temperature, the processing time, and the temperature and display time of the matrices should be taken into consideration.

On the basis of the modal cancer risk which is unacceptable de minimis, the study recommends an intervention to reduce the probable risk of cancer due to acrylamide exposure by the oral route.

This intervention should take into consideration other routes of exposure such as dermal and nasal.

On the basis of the sensitivity analysis, an effective and long term invention should target the processing methods with a view to reducing the level of risk by reducing the amount of acrylamide produced in the medium during processing. In this regard, cultivars and varieties of staples that store well with respect to low reducing sugar levels should help, as should actual reductive processing techniques.

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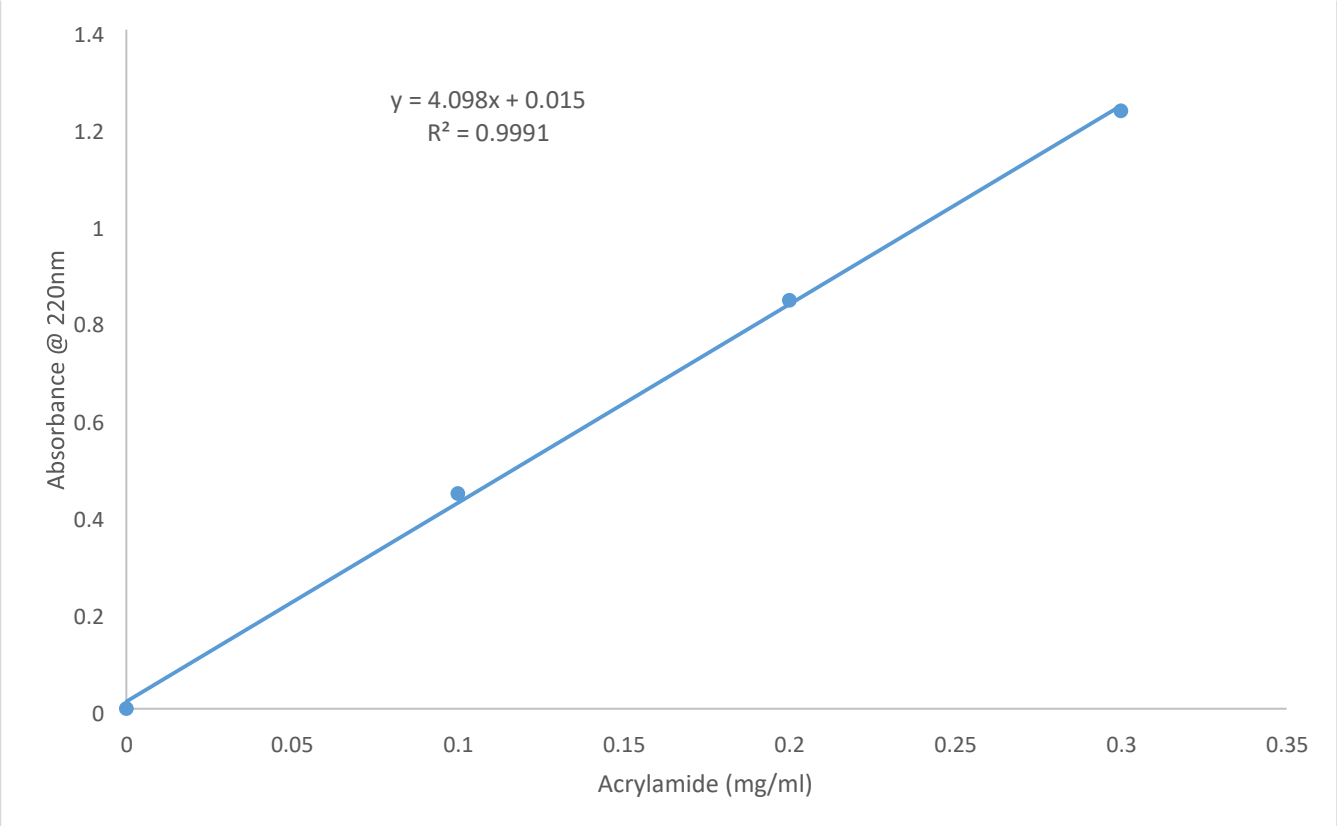
APPENDIX 1

ACRYLAMIDE CONCENTRATION CALIBRATION CURVE



APPENDIX 2

SAMPLE ACRYLAMIDE CONCENTRATION

Sample ID	Concentration/mg/g	Matrix
6A	2.8083	Biscuit
8A	4.3467	Biscuit
21A	2.6300	Biscuit
12B	0.5039	Biscuit
22B	0.8928	Biscuit
1B	0.6582	Biscuit
19A	2.5700	Biscuit
10B	0.4433	Buffloaf
19B	0.2721	Buffloaf
7	0.4423	Buffloaf
23	0.2671	Buffloaf
4B	0.4606	Buffloaf
23B	0.8574	Buffloaf
10A	1.2500	Chips

18A	2.2967	Chips
23A	2.7250	Chips
21B	0.4307	Chips
3C*	0.4081	Chips
3	3.6250	Fried fish
18	2.0825	Fried fish
16C	0.8388	Fried fish
21C	0.8712	Fried fish
23C	0.0512	Fried fish
11	2.7100	Fried fish
24A	1.5650	Fried plantain
11B	0.7662	Fried plantain
15B	0.3880	Fried plantain
25B	0.9291	Fried plantain
7A	2.5917	Fried plantain
16A	2.5717	Fried plantain
22A	1.6750	Fried plantain
9B	0.5210	Fried plantain
2	3.6175	Fried yam
13	2.8350	Fried yam
17	2.1850	Fried yam
24	2.9150	Fried Yam
8B	0.6857	fried yam
9C	0.3148	Fried yam
15C	0.1850	Fried Yam
1	2.5225	Groundnut
20	2.9763	Groundnut
1A	3.2600	Groundnut
3B	0.8986	Groundnut
16B	0.6426	Groundnut
10C	0.2615	Groundnut
17A	4.7883	Groundnut
14A	2.4625	Meat pie
20A	2.1400	Meat pie
13B	0.4069	Meat pie
2C	0.0470	Meat pie
4	3.3850	Roasted cocoyam
21	1.1354	Roasted cocoyam
7C	1.2751	Roasted cocoyam
14C	1.7590	Roasted cocoyam
18C	1.0369	Roasted cocoyam
5	0.0223	Roasted cocoyam
8	4.1550	Roasted plantain

22	3.2925	Roasted plantain
12A	2.2400	Roasted plantain
13A	2.7900	Roasted plantain
7B	0.4100	Roasted plantain
17B	0.6308	Roasted plantain
6C	0.6585	Roasted plantain
11C	0.7559	Roasted plantain
4A	2.4317	Roasted plantain
9	3.7590	Roasted yam
15	3.2850	Roasted yam
14B	0.4022	Roasted yam
4C	1.2354	Roasted Yam
17C	0.2751	Roasted yam
20C	0.6369	Roasted yam
16	3.3333	Smoked Tuna
25	3.3975	Smoked Tuna
15A	2.8833	Smoked tuna
18B	0.9204	Smoked tuna
8C	0.3154	Smoked Tuna
12C	0.3380	Smoked Tuna
22C	0.8028	Smoked Tuna
24C*	0.3062	Smoked Tuna
12	2.7200	Smoked Tuna
19	2.8475	Smoked Tuna

6	3.9169	Smoked Tuna
14	2.9488	Tea bread
11A	2.1883	Tea bread
5B	0.6085	Tea bread
6B	1.1152	Tea bread
20B	0.4018	Tea bread
5C	0.4661	Tea bread
19C	0.5887	Tea Bread

APPENDIX 3 DESCRIPTIVES

Weight of respondents taken by researcher in Kilograms

BW/kg	Frequency	Percent
12.32 - 26.97	14	2.2
26.98 - 41.64	74	11.8
41.65 - 56.31	236	37.5
56.32 - 70.98	221	35.1
70.99 - 85.65	64	10.2
85.66 - 100.32	16	2.5
100.33+	4	.6
Total	629	100.0

Marital Status and age of respondents in years

Age/years	Frequency	Percent
< 13	62	9.9
13+	567	90.1
Total	629	100.0

Marital status	Frequency	Percent
Single	494	78.5
Married 131 20.8 Divorced 4 .6		
Total	629	100.0

Level of Education and Current Employment Status

Employment status Frequency		Percent
Employed	267	42.4
Unemployed	91	14.5
Dependent	271	43.1
Total	629	100.0

Education	Frequency	Percent

Never been to formal school	30	4.8
Primary	85	13.5
Junior High School	247	39.3
Senior High School	214	34.0
Tertiary	53	8.4
Total	629	100.0

How Respondents Travel to Buy Food

How respondents travel to buy food	Frequency	Percent
Car	54	8.6
Walk	571	90.8
Bicycle	2	.3
Motor cycle	1	.2
Cook	1	.2
Total	629	100.0

Gender and location of respondents

Gender	Frequency	Percent
Male	353	56.1
Female	276	43.9
Total	629	100.0

Submetro	Frequency	Percent
Bantama	67	10.7
Nhyiaeso	62	9.9
Kwadaso	56	8.9
Oforikrom	64	10.2
Tafo	56	8.9
Suame	69	11.0
Asokwa	60	9.5
Subin	70	11.1
Manhyia	65	10.3
Asawase	60	9.5
Total	629	100.0

APPENDIX 4

QUESTIONNAIRE

I WEEK DIETARY RECALL

The questionnaire is about your eating habits for bread, meat pies, cakes, biscuits, pizza, chips, fried cocoyam chips, buffloaf, fried yam, fried plantain, fried fish, roasted ground nuts, ice creams, roasted maize, roasted plantain, roasted yam, roasted cocoyam, sausage and smoked fish, kelewele, •Answer each question as best you can. Estimate if you are not sure•A guess is better than leaving a blank•Put an X in the circle next to your answer.

Food type	In a week, number of times I eat								I usually buy								Weight	
	Never	1 time	2 times	3 times	4 times	5 times	6 times	7 times	Never	<¢1	¢1 - ¢2	¢2 ¢3	¢2 - ¢3	¢3 ¢4	¢3 - ¢4	¢4>	Weight per ¢1	
Roasted maize																		
Roasted plantain																		
Roasted yam																		
Roasted cocoyam																		
Roasted groundnuts																		
sausage																		
smoked fish																		
bread																		
meat pie																		
cake																		
biscuit																		
pizza																		
chips																		
fried cocoyam chips																		
fried yam																		
fried plantain																		
fried fish																		
buffloaf																		
kelewele																		
ice cream																		

Weight of respondent(Kg) taken by researcher:

1. Are you:

☐ Male

☐ Female

2. What is your age?

_____

3. How did you travel here?

☐ Car

☐ Plane

☐ Train

☐ Bus

☐ Other (please specify: .....)

4. You have schooled up to

☐ Never been to formal school

☐ Primary

☐ Junior High

☐ Senior High

☐ First degree

☐ Second degree

☐ Doctoral/post doctoral

Other (please specify: .....)

5. Are you

☐ Employed,

☐ Unemployed or

☐ Dependent.

6. Are you

☐ Single,

☐ Married or

☐ Divorced.