University of Cape Coast

UNIVERSITY OF CAPE COAST

Investigation into Iodine Deficiency of Selected Populations within Cape Coast Metropolis and Agona West Municipality in the Central Region of Ghana – Analysis of Iodised Edible Salts and Urine.

BY

BENJAMIN BARTELS

Thesis submitted to the Department of Chemistry, School of Physical Sciences of the College of Agriculture and Natural Sciences, University of Cape Coast, in partial fulfilment of the requirements for the award of Doctor of Philosophy degree in Chemistry

MARCH 2019

DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original research and that no part of it has been presented for another degree in this university or elsewhere.

Candidate's Signature…….. Date…….....

Name: Benjamin Bartels

Supervisor's Declaration

I hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

Supervisor's Signature…….. Date ……....

Name: Professor Victor Patrick Yao Gadzekpo

ABSTRACT

The iodine status of staff and pupils of Hillcrest Schools, undergraduates of UCC, 2016/2017 academic year and a household in Agona Swedru were determined using Titration Method, Sandell-Kolthoff Method and a Novel Method. Discriminant analysis results showed that the Novel Method correctly classified 77.4% of the original cases. The novel also had a percentage recovery of125% and a Horwitz Ratio of 0.12. Based on these, the study populations with the exception of household, were found to be iodine sufficient by the Novel Method.The iodine level in the Buffalo was 10 ppm; Ritebrand was non-detect. These levels were below the Ghana Standard Authority's limit of 25-50 ppm. The stability of the iodine in the salt was found to be influenced by magnesium content, moisture content and heat. Pb levels found in Annapurna, U2 and Ante Dede were respectively 51, 13.31 and 3.4 ppm. These levels were above the Codex limit of 2 ppm. The concentration of Fe in Mr. Chef, U2, Lele and Ante Dede were respectively 0.95, 0.49, 0.58 and 0.25 ppm. However, the concentrations of Zn were non-detect in all the samples. The Cd levels in Concord, Salnova and Cerebos iodised salt with 0.20, 0.05, and 0.01 ppm respectively, below the Codex limit of 0.5 ppm. Al levels were non-detect for all the salt samples. Microbial studies revealed Annapurna Salt contained microbes. Consumption of goitrogens, preference for uniodised salts, ignorance about the mandatory salt iodisation law, and iodine status test were behavioural patterns found to have the potential of causing iodine deficiency in the study populations.

KEY WORDS

Iodine Deficiency Disorder

Iodised Salt

Novel

Spectrophotometer

Survey

Urine

ACKNOWLEDGEMENTS

I am indebted to Professor Victor Patrick Yao Gadzekpo, FGA, who is my supervisor and then Associate Professor Ruphino Zugle, and all staff of the Department of Chemistry, University of Cape Coast

I am also grateful to Associate Professor John Kwesi Bentum, Dr. Joseph Adjei, Dr. Samuel Tetteh, Dr. Albert Ofori, all of Chemistry Department and Associate Professor Frederick Ato-Armah of the University of Cape Coast for their diverse assistance.

I am again grateful to Mary Bentil for typing the work.

DEDICATION

To Professor Victor Patrick Yao Gadzekpo, FGA and Mrs. Dedina Victoria Gadzekpo

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ACRONYMS

WHO	World Health Organization
IDDs	Iodine Deficiency Disorders
ICCIDD	International Council for the Control of Iodine Deficiency Disorders
USI	Universal Salt Iodisation
MUIC	Median Urinary Iodine Concentration
GOG	Government of Ghana
UIC	Urinary Iodine Concentration
NAAS	Neutron Activation Analysis Instrument
ISE	Ion Selective Electrode
DRI	Dietary Reference Intake
FNB	Food and Nutrition Board
RDA	Recommended Daily Allowance
AI	Adequate Intake
EAR	Esimated Average Requirement
UL	Tolerable Upper Intake Level
ACE	Angiostensins – Converting Enzymes
NRC	National Research Council
TSH	Thyroid Stimulating Hormone
T4	Thyrosine (tetraiodothyronine)
T3	Triodothyronine
NIS	Sodium-Iodine Sympoter
SHBGT	Sex-Hormone Binding Globulin
NOAEL	No-Observed-Adverse-Effect Level
MRL	Minimal Risk Level

TPA Thyroid Peroxidase antibodies

LOAEL Lowest-Observed-Adverse-Effect Level

GSA Ghana Standard Authority

UNICEF United Nations Children Education Fund

ATSDR Agency for Toxic Substances and Disease Registry

ANOVA Analysis of Variance

CHAPTER ONE

INTRODUCTION

The chapter captures the attempt by World Health Organisation (WHO) to solve global iodine deficiency and its disorders. It also gives insight into the contributions, failures and successes of the Government of Ghana (GOG), in eliminating iodine deficiency disorders (IDD). The importance of the study section illucidated causes of IDD in Ghana, and also highlighted the specific objectives and hypotheses for the study.

Background to the Study

In 1960, World Health Organisation (WHO) alerted the international community concerning public health importance of iodine deficiency (Zimmermann, 2008). Then in 1990, it adopted universal salt iodisation (USI) as the strategy of choice for controlling global IDD. Consequently, a national survey in 1992 showed incidents of IDD in Ghana. As a result, Ghana adopted the strategy and passed a law to make iodisation of salt mandatory in 1996 (National Iodine Survey Report *GHANA*, 2015).

In 2004, the GOG embarked on effective iodisation of all salt. It also monitored and enforced the salt iodisation law; and increased national awareness of the benefits of iodised salt (National Iodine Survey Report *GHANA* 2015).

Inspite of these efforts, thyroid cases continued to increase. The increase was attributed to inadequate iodisation of salt; and behavioral patterns of consumers (Chirawurah et al., 2014; Asibey-Berko et al., 2014; Buxton et al., 2012). The GOG then conducted another survey between 2009-2010, this

also showed an increase in IDD (National Iodine Survey Report *GHANA* 2015).

The recent national survey has established a national adequacy in iodine status. It also reported substantial improvement since the 2009-10 survey (National Iodine Survey Report *GHANA* 2015). However, Sarfo – Kantanka et al., (2017) and Aryee, (2018) had reported prevalence of the disease in the Central Region of Ghana.

Statement of the Problem

IDD were expected to be eliminated in Ghana by 2005. Yet, successive national surveys until 2010 indicated an increase. Even though the 2015 survey showed a substantial improvement of the situation, there are still populations that are vulnerable according to National Iodine Survey Report 2015.

Previous findings on IDD (Gbadegbo & Nwufoh, 2010) have established a relationship between iodine intake and urinary iodine. It has also been established that about 90% of ingested iodine is excreted in the urine; and that urine is an excellent biomarker for recent iodine consumption (WHO A Guide for Programme Managers, 2014).

Justification of the Study

Though recent survey in 2015 showed a national adequacy in iodine status, a significant increase in thyroid admissions in central Ghana over the decades, and a progressive increase in the prevalence has been reported by Der et al., (2013); Sarfo-Katanka et al., (2017); and Aryee et al., (2018). According to report by Ghana Nutrition Profile 2011, only 32% of iodised salt is properly iodized in Ghana, 3% of children in Ghana are cretins, 10% are severely

mentally impaired, 87% mildly mentally impaired and their intelligence reduced permanently by 13.5 IQ points. Again, 1.5 million of these children have also been reported to be affected by mild to severe mental impairment from 2011-2020; and productivity losses in this period shall be 5,974 million Ghana cedis (Ghana Nutrition Profiles, 2011)

Importance of the Study

It shall provide emperical data on urinary iodine of respondents; and the levels of iodine in iodised salt. Such information shall provide knowledge in developing policies to solve iodine deficiency issues.

Hypotheses of the Study

Urinary iodine

If there is no prevalence of iodine deficiency in the study population then the median urinary iodine concentration found for the population should be within 100 - 199 ppm of the WHO epidemiological range or 650 – 869 ppm of the novel epidemiological range.

Salt iodine content

If the iodised salt sold in Ghana is adequately iodised and capable of eliminating iodine deficiency then the iodine concentration found therein should be within the legal limit of 25-50 ppm set by Ghana Standard Authority.

Objectives of the Study

General Objective

Analyse the amount of iodine in iodised salt and in urine to determine the incidents of iodine deficiency in the study populations; and also predict the behavior of the respondents that could cause the deficiency. The questionnaire

gathered information about iodised salts, iodine deficiency and other relevant information that could not be determined experimentally.

Specific Objectives

The specific objectives are to:

1. Analyse the concentration of urinary iodine of the study population and to establish their iodine nutrition status.
2. Analyse iodised salt samples (packaged and unpackaged) for their concentration of iodine to ascertain if the study population is consuming adequate iodised salt.
3. Determine the stability of iodine in the salt to assess the factors that causes inadequacy of salt iodine under local conditions.
4. Undertake comparative study of the analytical methods for urinary iodine determination in this study,
5. Determine the behaviorial patterns responsible for the iodine deficiency or iodine sufficiency of the study population.

Delimitation

These included the choice of objectives, research questions, variables of interest, the study population, criteria of participants, the geographic region, and the selected methodology and variables. Specifically, urine was preferred because it is readily available, sterile and about 90% of the ingested iodine is excreted in it (A Guide for Programme Managers, 2004).The study populations were chosen because of proximity and acessibility.

Scope of the Study

Salt iodine content of salt samples collected across Ghana were determined to establish their adequacy in iodine. Then, urine samples (ethical

clearance obtained from IRB-UCC, Appendix ZS) of the study populations were also analysed for their iodine, the median urinary iodine concentrations were used to determine their iodine status. Questionnaire responses were used to evaluate the behaviorial changes that are responsible for iodine deficiency in the populations. An attempt was made to develop an improved method to determine the urinary iodine concentration.

The salt iodine content was determined by iodometric method as recommended by WHO/ICCIDD, the metal content of the salt was determined by spectrophotometric method, and then microbial and iodine stability of the the salt were also studied.

Limitations

The factors that limited the scope were:

1. Difficulty in obtaining reference urine sample and standard reference materials
2. Lack of cooperation and outright refusal by certain state agencies to provide information on the current state of IDD in Ghana compelled me to solely depend on textbooks, published articles, questionnaire and the internet for information.
3. Non availability of technologically advanced equipment like Neutron Activation Analysis Instrument (NAAS) and Ion Selective Eectrode (ISE) for analysis forced me to rely on other methods though limited in sensitivity but recommended for the analysis by UNICEF/WHO.

Structure of the Thesis

The thesis was structured in five chapters. Chapter one covered the introduction which provided the rationale for the study and gave the scope and

nature of the problem. It indicated the background to the study, the problem statement, objectives and relevance of the study. It also covered the scope, limitation, delimitation and hypotheses of the study. Chapter two discussed relevant literature on iodine deficiency disorders; and threat to human lives, and the various approaches used in controlling it. It also reviewed the approach, the principal results and conclusions from the study.

Chapter three covered methods and materials for sampling, data collection, questionnaire administration, and the analytical methods; whilst chapter four included the analytical results, the findings, and discussions of same, and observations.The discussion showed the relationships among the facts and put the results in context of previous research. It also showed the trends, and generalizations of the results. In chapter five, the principal findings were summarised; and conclusions and recommendations made.

Assumptions of the Study

1. That the questionnaire was honestly answered since the respondent's identity was kept anonymous and were also told they could leave anytime without any hindrance.
2. That the urine samples collected were not contaminated since they were handled by competent hospital staff and collected into sterilized vials.

Chapter Summary

The causes of iodine deficiency disorders have been outlined. Recent reports about the prevalence of these disorders in Ghana (Chirawurah et al., 2015; Buxton, 2012; Abu et al., 2018) have also been captured. The enormous health issues associated with consumption of uniodised salt; lack of

knowledge about iodine status; and the mandatory salt iodisation law have also been captured.

CHAPTER TWO

LITERATURE REVIEW

Introduction

This chapter explores literature about the cause and effect of IDDs and the efforts of International Organizations like WHO/UNICEF and ICCIDD and Government of Ghana in eliminating IDDs. It will also present review and critique of analytical methods employed in this study to determine the urinary iodine concentration. As set out chapter one, the objective of this study is to determine the levels of iodine in human urine and in edible salt; and also the behavioural patterns of respondents to assess the causes of IDDs in Ghana.The chapter also aims at establishing the iodine status of the populations to ascertain whether it is within the optimal nutrition range of 100-199 or 650 – 869 ppm.

It will also establish salt iodine concentration at the retail outlet to assess its compliance of the mandatory 25-50 ppm iodine level set by GSA. Also, a comparative study of the selected analytical methods will be carried out. Questionnaire information will be used to determine the behavioural patterns of respondents toward eliminating IDD in Ghana.

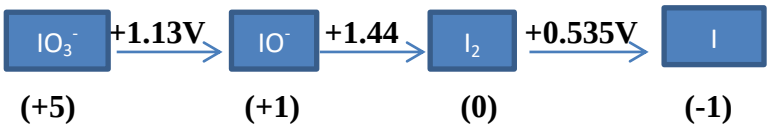
Physicochemical Properties of Iodine

Iodine is a nonmetallic element. It belongs to the halogen family in Group VIIA of the periodic table. It is found in nature as iodide, I^- ; or in molecular compounds with other elements, for example, IO_3^-. The element is a simple electron short of noble gas structure, for this reason the dominating feature of its chemistry is the ease with which its atoms acquire an electron to become uninegative ions. The noble gas structure causes it to become stable:

$I_{2\,(g)} + e \longrightarrow 2I^-_{(aq)}$ .....(1)

$I_{2\,(aq)} + e \longrightarrow 2I^-_{(aq)}$.....(2)

Iodine can exist in several oxidation states in acidic or basic conditions. In acidic conditions:



Chronology of Iodine Deficiency Disorders

In 1820, IDD as a risk factor for goiter was discovered. Then in 1895 iodine was discovered in the thyroid gland by Baumann. In 1914, Kendall isolated from thyroid gland a pure crystalline substance containing about 65% of iodine, that he named thyrozine and established its effects on metabolism. Consequently, Paul Marine in 1916 conducted an experiment in Ackron/Ohio, USA, in which two groups of school children were treated to iodised salt, in the treated group two people developed goiter. Prior to this Michigan school children of USA had 47% goiter incidence at that period (Iodine Status Worldwide, 2004).

From 1920 to 1931 methodologies for determining iodine in matrices intensified, and in the same period Switzerland and USA embarked on large scale salt iodisation. Notably, USA adopted the use of iodised salt in 1924. Harrington determined the detailed structure of thyrozine in 1926 whilst Forster isolated a compound 3, 5-diidotyrosine from thyroid an 1929. In 1930, it was established that iodine sufficiency of 150 ppm is required to eliminate IDDs (Iodine Status Worldwide, 2004).

Heidelberger et al (1935) determined the molecular weight of thyrosine. In the same year, Marine reviewed the function of the thyroid gland,

and estimated a maximum iodine content of 20-25 mg in human body, the average being 10-15 mg. In 1949 (Iodine Status Worldwide, 2004).

In 1983, 400 million IDD were discovered in less developed countries. Seven years later, in 1990 World Summit for Children was held, and subsequently, World Health Assembly resolved to eliminate iodine deficiency as public health problems globally in 1991. As a result, International Conference on Nutrition postulated elimination of IDD by 2000 in 1992. In 1992, Kazakhstan legislated the use of iodised salt (Iodine Status Worldwide, 2004).

In 1993, it was established that 110 countries had IDD. A year of Universal Salt Iodisation (USI) was declared in 1995. In South Africa the government instructed salt iodisation in 1995 (Sheohua, 2000). In the same year Ghana, legislated iodised salt. In 2003, 54 countries had IDD, this figure dropped to 47 in 2007 and then in 2012 it further decreased to 30 countries. In 2015, it decreased further to 25 countries. Various analytical methods have been tried in the 21st century to analyse the iodine in salt and in urine (Iodine Status Worldwide, 2004).

Recommended Intakes of Dietary Iodine

These RDA which may vary by age and gender, include:

Recommended Daily Allowance (RDA), Adequate Intake (AI), Estimated Average Requirement (EAR), and Tolerable Upper Intake Level (UL).

Average Daily Allowance

Average Daily Allowance which is sufficient to meet the nutrient requirements of nearly all (97-98%) healthy individuals are shown in Table 1.

Table 1: Recommended Daily Allowance (RDAs) for Iodine

Age	Male	Female	Pregnancy	Lactation
Birth to 6 mths	110*	110*		
7-12 mths	130*	130*		
1-3 yrs	90	90		
4-8 yrs	90	90		
9-13 yrs	120	120		
14-18 yrs	150	150	220/250	290
19+ yrs	150	150	220/250	290

Source: Institute of Medicine, 2001; WHO A Guide for Programme Managers, 2007.

* Adequate Intake (AI)

Adequate Intake (AI)

Established when evidence is insufficient to develop an RDA and it is set at a level assumed to ensure nutritional adequacy.

Estimated Average Requirement (EAR)

Average daily level of intake estimated to the requirements of 50% of healthy individuals. It is usually used to assess the adequacy of nutrient intakes in populations but not individuals.

Tolerable Upper Intake Level (UL)

Maximum daily intake unlikely to cause adverse health effects (Institute of Medicine, 2001), Table 2.

Table 2: Tolerable Upper Intake Levels (UIs) for Iodine

Age	Male	Female	Pregnancy	Lactation
Birth to 6 mths	Not possible to establish	Not possible to establish		
7 -12 mths	Not possible to establish	Not possible to establish		
1-3 yrs	200 μg	200 μg		
4-8 yrs	300 μg	300 μg		
9-13 yrs	600 μg	600 μg		
14-18 yrs	900 μg	900 μg	900 μg	900 μg
19+ yrs	1,100 μg	1,100 μg	1,100 μg	1,100 μg

Source: Institute of Medicine, 2001
*Formula and food should be the only sources of iodine for infants

Dietary Sources of Iodine

Iodine is added to food, such as table salt, to ensure that there is enough iodine in the body to form essential thyroid hormones. Iodine is put into animal feeds for the same reason (ATSDR, 2004a).

Iodine in iodised salt is present as sodium and potassium salts, such as iodate (IO_3^-) and iodide (I^-), the reduced form of iodine (Patrick, 2008). The iodate is quickly and almost completely absorbed in the stomach and duodenum. It is reduced in the gastrointestinal tract and absorbed as iodide (Institute of Medicine, 2001; Zimmerman, 2009). When iodide enters the circulation, the thyroid gland concentrates it in appropriate amounts for thyroid hormone synthesis. Most of the remaining amount is excreted in the urine (Institute of Medicine, 2001). The iodine-replete healthy adult has about

15-20 mg of iodine, 70-80% of which is contained in the thyroid (Zimmerman, 2008).

Iodine is also found in certain foods such as seaweed, which is one of the best food sources of iodine. Other good sources include seafood, dairy products, grain products, and eggs (Murray et al., 2008). Other sources include certain fish and shell fish and processed foods (Harvard Heart Letter, 2011),

Fruits and vegetables also contain iodine, the concentration ranges between 10 μg/kg to 1 mg/kg dry weight (Zimmerman, 2008). This variability in turn affects the iodine content of meat and animal products because it affects the iodine content of foods that the animals consume (Zimmerman, 2008).

In areas where people and drinking water are constantly receiving the iodine-containing salt spray, the intake of iodine is presumably adequate, and in such regions goiter is very rare. In regions too remote or too mountainous to receive significant amounts either directly or indirectly from the sea, goiter is much more common, as in the Great Lakes region and much of the Northwest of the US, in parts of Switzerland and in several other parts of the world (Iodine Status Worldwide, 2004). Other sources include iodised salt (Dalia et al, 2017).

Iodine and Health

Iodine is needed by the thyroid gland to produce thyroid hormones. The thyroid gland is healthy when there is just enough iodine in the body, about 10-15 mg, so that just the right amounts of thyroid hormones are produced. The thyroid gland can become unhealthy if more or less than this amount of iodine is in the body. An unhealthy thyroid gland can affect the

entire body. If the thyroid gland cannot make enough hormones, then one would have to be given thyroid hormone in pills. If the thyroid gland makes too much hormone, then you would have to be given drugs to make the thyroid make less hormone (ATSDR, 2004).

Babies and children need iodine to form thyroid hormones too, and just the right amount of iodine from mothers before being born. Too much iodine from the mother can cause a baby's thyroid gland to be so large that it makes breathing difficult or impossible. Not enough iodine from the mother can cause a baby to not produce enough thyroid hormone, which can affect growth and mental development of the baby. Children are more sensitive to the harmful toxic effects of iodine than adults because their thyroid glands are still growing and the thyroid gland tissues are more easily harmed (ATSDR, 2004). Severe maternal iodine deficiency lead to mental and growth retardation or cretinism offspring, and even mild maternal iodine deficiency has been associated with lower IQ in children.

Due to its important role in fetal and infant development and thyroid hormone production, iodine is a critical nutrient for proper health at the life stages especially in fetal and infant development, cognitive function during childhood, prevention of fibrocystic breast disease, and radiation-induced thyroid cancer (Zimmerman, 2008; Melse-Boontra & Jasural, 2010; Kessler, 2014; Center for Drug Evaluation and Research, 2001). High intakes of iodine can cause some of the same symptoms as iodine deficiency including goiter, elevated TSH levels, and hypothyroidism because excessive iodine (overabundance) in susceptible individuals inhibits thyroid hormone synthesis and thereby increases TSH stimulation which can produce goiter. Iodine-

induced hyperthyroidism can also suit for high iodine intakes, usually when iodine is administered to treat iodine deficiency. Acute poisoning symptoms include burning of the mouth, throat, and stomach, fever, abdominal pain, nausea, vomiting, diarrhea, weak pulse and coma (Institute of Medicine, 2001). People with autoimmune thyroid disease and iodine deficiency, may experience adverse effects with iodine intakes considered safe for the general population (Institute of Medicine, 2001).

The Food and Drugs Board (FNB) of USA has established iodine levels for food and supplement intakes from foods and supplements are unlikely to exceed the level (Institute of Medicine, 2001). Long-term intakes above the level increase the risk of adverse health effects. The levels do not apply to individuals receiving iodine for medical treatment, but such individuals should be under the care of physician (Institute of Medicine, 2001).

Iodine supplements have the potential to interact with several types of medications; there are anti-thyroid medications, Angiostensins–Converting Enzyme (ACE) inhibitors, and potassium-sparing diuretics (Natural-Medicine Comprehensive Database, 2009). Nutritional needs should be met primarily from foods containing vitamins, and minerals and dietary fibre and other naturally occurring substances.

Relevance of Iodine to Public Health

The term “iodine excess” is used to refer to increases in intake relative to estimated physiological requirements. As a reference point, the chronic dietary intake of iodine in U.S populations has been estimated to range from 150-950 µg/dy. Estimates for various populations have ranged from <50 µg/dy

in iodine-deficient regions to more than 10 mg/dy in populations that regularly ingest seaweeds containing a high iodine content. The National Research Council (NRC) of USA Recommended Dietary Allowance (RDA) for iodine is 150 µg/dy (2.1 µg/kg/dy for a 70-kg adult), with additional allowances of 25 and 50 µg/dy during pregnancy and lactation, respectively. The diet is the major source of iodine intake in the U.S population. Iodine enters the human diet from a variety of natural sources, including mineral dissolution and atmospheric transport and deposition of seawater aerosols to surface water, vegetation, and soil. Major food categories that contribute to dietary iodine include marine produce (for example, fish and shellfish) and milk. Cows and goats absorb iodine from ingested vegetation and water, when iodine is either deposited on the vegetation or in water or when the iodine is taken up by vegetation grown in soils containing iodine.

The absorbed iodine is excreted into their milk; goat milk typically has higher concentrations of iodine than cow milk for equal deposition on feed. Additional sources of iodine in milk derive from the use of iodine disinfectants on cows, milking machines, and other milk processing equipment, as well as from supplementation of dairy feed with iodine-containing compounds. Breast milk is the primary source of iodine intake in nursing infants. Commercial infant formula preparations are fortified with sufficient iodine to support infant health, growth, and development. Cow milk is a significant source of iodine intake in children. Iodine is also intentionally added to the U.S. diet as iodised table salt and as iodine-containing bread dough oxidizers. Other sources of intake derive from the use of iodine-containing topical disinfectants (for example, povidone iodine), iodine-containing diagnostic and therapeutic

agents, dietary supplements, and water purifiers containing iodine (ATSDR, 2004).

Risk Factors of Iodine Deficiency

The following are potential risk factors that may lead to iodine deficiency: low dietary iodine, selenium deficiency, pregnancy, exposure to radiation, increased intake/plasma level of goitrogens, such as calcium, thiocyanate, perchlorates, gender (higher occurrence in women), smoking tobacco, alcohol, oral contraceptives, and age (for different types of iodine deficiency at different ages) (Knudsen et al., 2001). With iodine supplementation, goiters caused by iodine deficiency (determine in size in very young children and pregnant women), respond with only small amounts of shrinkages, and patients are at risk of developing hyperthyroidism (Stephanie, 2014). Following the adoption of iodised salt in the US in 1924 there was a gradual increase in average intelligence of 1 standard deviation, 15 points in iodine-deficiency areas, 3.5 points nationally, but also an increase in deaths of older people in iodine-deficient areas due to hyperthyroidism (Max, 2013; Stephanie, 2014).

A general idea of whether a risk or deficiency exists can be determined through a functional iodine test, (Lord, 2008; Richard, 2008). Minimal Risk Levels (MRLs) for stable iodine (^{127}I) is based on an assessment of dose-response relationships for the chemical toxicity of stable iodine. An MRL of 0.01 mg/kg/dy has been derived for acute-duration oral exposure (1-14 dys) to iodine. The acute-duration MRL is based on a no-observed-adverse-effect-level (NOAEL) of 0.01 mg/kg/dy in healthy adult humans. Although the NOAEL is derived from acute studies of healthy adults, supporting studies

indicate that the NOAEL would also be applicable to children and elderly adults. On this basis, an uncertainty factor is not needed adjust the NOAEL to account for human variability in sensitivity.

Based on 24-hr urinary excretion of iodide prior to the iodide supplement, the background iodine intake was estimated to be approximately 200 µg/dy, thus, the total iodide intake was approximately 450, 700, or 1,700 µg I/dy (approximately 0.0064, 0.01, or 0.024 mg/kg/dy, respectively, assuming a 70-kg body wt). Subjects who received 1,700 µg/dy (0.024 mg/kg/day) had significantly decreased (5-10%) serum concentrations of TT4, FT4, and TT3 compared to pretreatment levels, and serum TSH concentrations were significantly increased (47%) compared to pretreatment values. All hormone levels were within the normal range during treatment. In this same study, the subjects who received daily doses of 250 or 500 µg I/dy for 14 dys (respective total intakes of approximately 450 or 700 µg/dy; 0.0064 or 0.010 mg/kg/dy) had no significant changes in serum hormone concentrations. A limitation of this study is that it included a relatively small number of subjects, although the exposures to these subjects were controlled and quantified with high certainty (ATSDR, 2004).

Distribution and Metabolism of Iodine in Adult

The body of the average adult contains a small amount of the trace element iodine, from 20-50 mg approximately 50% of this is in the muscles, 20% in the thyroid glands, 10% in the skin, and 6% in the skeleton. The remaining 14% is scattered in other endocrine tissue in the central nervous system, and in plasma transport (Zimmermann, 2008). Iodine accounts for 65% of the molecular weight of T4 and 59% of the T3. 15-20 mg of iodine is

concentrated in thyroid tissue and hormones, but 70% of the body's iodine is distributed in other tissues-including mammary glands, eyes, gastric mucosa, choroid plexus, arterial walls, the cervix, and salivary glands (Zimmermann, 2008). In the cells of these tissues, iodine enters directly by sodium-iodide symporter (NIS). The human body contains approximately 10-15 mg of iodine, of which approximately 70-90% is in the thyroid gland, which accumulates iodine in producing thyroid hormones for export to the blood and other tissues (Hays, 2001). The concentration of iodine in serum is approximately 50-100 μg/L under normal circumstances (Fisher et al., 2011). Approximately 5% in serum is in the inorganic form as iodide; the remaining 95% consists of various organic forms of iodine, principally protein complexes of the thyroid hormones T4 and T3 (ATSDR, 2004). The tissue distribution of iodide and organic iodine are very different and are interrelated by metabolic pathways that lead to the iodination and deiodination of proteins and thyroid hormones in the body. Other tissues that can accumulate iodide to a concentration greater than that of blood or serum include the salivary glands, gastric mucosa, choroid plexus, mammary glands, placenta, and sweat glands (ATSDR, 2004). Iodide taken up by the thyroid gland is utilized in the production of thyroid hormones, which are stored in the gland. This organic fraction of the thyroid iodine content accounts for approximately 90% of the iodine in the thyroid gland and includes iodinated tyrosine and tyrosine residues that comprise the thyroid hormones, T4 and T3, and their various synthesis intermediates and degradation products (ATSDR, 2004). Nearly all (>99 %) of the T4 and T3 in plasma are bound to protein. The major binding

protein for T4 and T3 is thyroxine-binding globulin (TBG), which has a high affinity for both hormones, Table 3.

Table 3: Binding Characteristics of Major Human Thyroid Hormone- Binding Proteins

Parameter	Thyroxin-blinding globulin	Transthyretin	Albumin
Molecular weight of complex (D)	54,000	54,000 (subunit)[a]	66,000
Plasma concentration ($\mu gT_4/dL$)	0.27	4.6	640
T_4 blinding capacity ($\mu gT_4/dL$)	21	350	50,000
Association constant (M^{-1})			
T_4	$1x10^{10}$	$7 x10^7$	$7 x10^5$
T_3	$5 x10^8$	$1.4 x10^7$	$1 x10^5$
Fraction of sites occupied by T_4[b]	0.31	0.02	<0.001
Distribution volume (L)	7	5.7	7.8
Turnover rate (percent/day)	13	59	5
Distribution of thyronines (percent/protein)			
T_4	68	11	20
T_3	80	9	11

Source: ATSDR, 2004

[a]Transthyretin consists of four subunits (54 kD) complexed with retinol binding protein [b]In euthyroid state T3=3,5,3N-triiodo-L-thyronine; T4=3,5,3N,5N-tetraiodo-L-thyronine (thyroxine)

Other proteins that bind thyroid hormones, with lower affinity, include transthyretin (thyroxine-binding prealbumin), albumin, and various apoproteins of the high density lipoproteins HDL2 and HDL3 (3-6% of plasma hormones). The distribution of protein-bound thyroid hormones is largely confined to the plasma space, whereas the free hormones distribute to the intracellular space of a wide variety of tissues where they exert the

metabolic effects attributed to thyroid hormones. TBG and other binding proteins serve as reservoirs for circulating thyroid hormones and contribute to the maintenance of relatively constant free hormone concentrations in plasma. Uptake of T4 and T3 into liver, skeletal muscle, and other tissues occurs by a saturable, energy-dependent carrier transport system. Lipoprotein transport mechanisms may also play a role in the uptake of thyroid hormones into certain tissues .Intracellular T4 and T3 exist as free hormone and are bound to a variety of intracellular proteins. Maternal exposure to iodine results in exposure to the fetus. Radioiodine accumulation in the fetal thyroid commences in humans at approximately 70-80 dys of gestation, and precedes the development of thyroid follicles and follicle colloid, which are generally detectable at approximately 100-120 dys of gestation (ATSDR, 2004). Fetal iodide uptake activity increases with the development of the fetal thyroid and reaches its peak at approximately 6 mths of gestation, at which point, the highest concentrations in thyroid are achieved, approximately 5% of the maternal dose/g fetal thyroid (approximately 1% of the maternal dose) (ATSDR, 2004). Fetal radioiodine concentrations 1-2 dys following a single maternal dose of radioiodine generally exceed the concurrent maternal thyroid concentration by a factor of 2-8 with the highest fetal/maternal ratios occurring at approximately 6 months of gestation (Millard et al., 2001). Following long-term exposure, either from ingestion of administered radioiodine or from exposure to radioactive fallout, the fetal/maternal ratio for thyroid radioiodine concentration has been estimated to be approximately 2-3 (ATSDR, 2004).

Iodine uptake into the thyroid gland is highly sensitive to the iodide intake. At very low intakes, representing iodine deficiency (for example, 20μg/dy), uptake of iodide into the thyroid gland is increased. This response is mediated by TSH, which stimulates iodide transport and iodothyronine production in the thyroid gland.The fraction of an ingested (or injected) tracer dose of radioiodide that is present in the thyroid gland 24 hrs after the dose has been measured in thousands of patients who received radioiodine for treatment of various thyroid disorders or for the assessment of thyroid function; these provide a comparative index of effects of various factors on the distribution of absorbed iodide to the thyroid gland. A single oral dose of 30 mg iodide (as sodium iodide) decreases the 24-hr thyroid uptake of radioiodine by approximately 90% in healthy adults. The inhibition of uptake was sustained with repeated oral doses of sodium iodide for 12 dys, with complete recovery to control (presodium iodide) uptake levels within 6 weeks after the last sodium iodide dose or within 8 dys after a single dose. Repeated oral doses of 1.5-2.0 mg I/m^2 of surface area produced an 80% decrease in thyroid uptake in children.

Twenty-four-hour radioiodine uptakes into the thyroid gland in males and females who experience similar iodide intakes are similar, although uptakes in females, as a percentage of the dose, appear to be 10-30% higher than in males. Thyroid uptakes in newborns are 3-4 times greater during the first 10 dys of postnatal life than in adults, and decline to adult levels after approximately age 10-14 dys (ATSDR, 2004).

Iodide in the thyroid gland is incorporated into a protein, thyroglobulin, as covalent complexes with tyrosine residues, Figure 1.

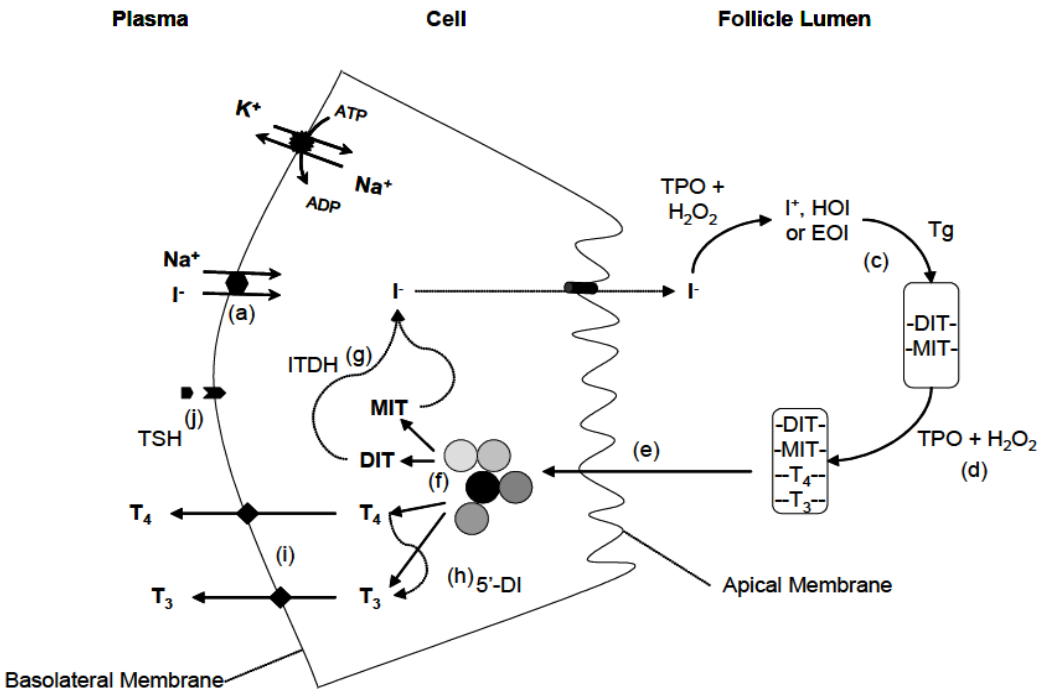


Figure 1: Pathways, Uptake and Metabolism of Iodide in the Thyroid Gland. Source: ATSDR, 2004

The iodination of thyroglobulin is catalyzed by the enzyme thyroid peroxidase, which resides predominantly in the apical membrane of thyroid follicle cells, with the active sites of the enzyme facing the follicular lumen. The iodination reactions occur at the follicular cell-lumen interface and consist of the oxidation of iodide to form a reactive intermediate, the formation of monoiodotyrosine and diiodotyrosine residues in thyroglobulin, and the coupling of the iodinated tyrosine residues to form T4 (coupling of two diiodotyrosine residues) or T3 (coupling of a monoiodotyrosine and diiodotyrosine residue) in thyroglobulin, Figure 2.

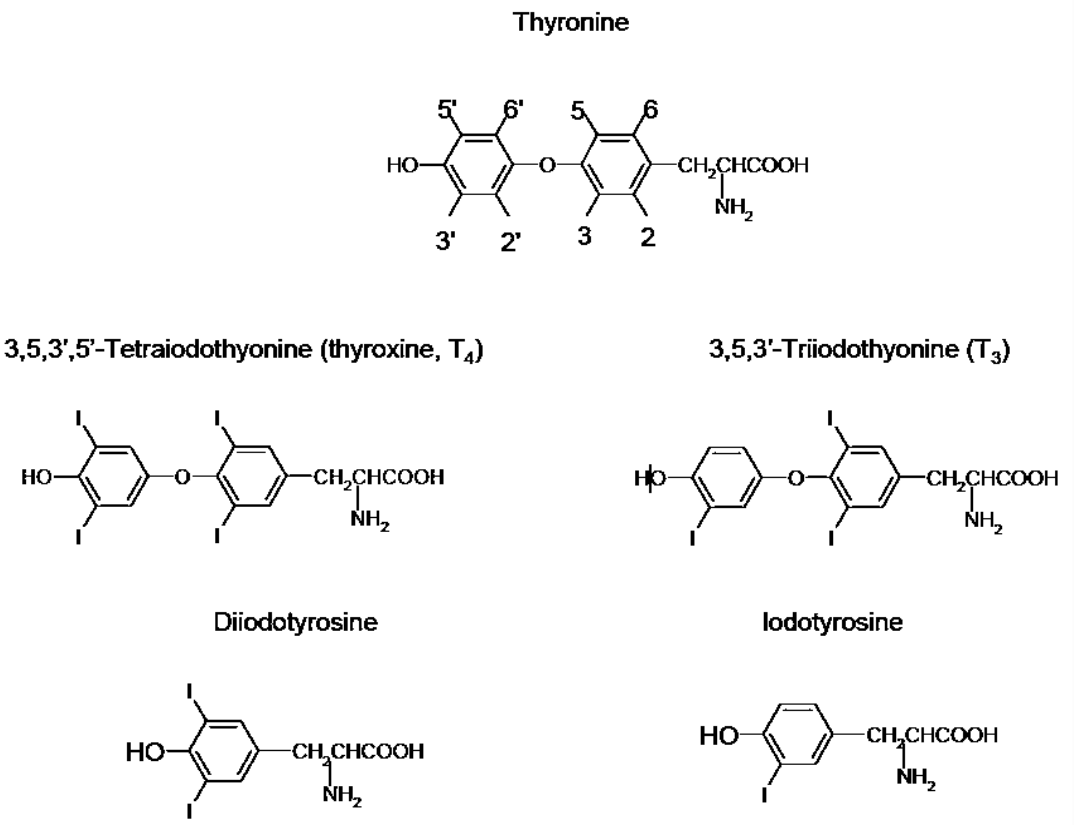


Figure 2: Formation of iodinated tyrosine residues.

The Metabolism of Iodine

A variety of chemical inhibitors of iodine thyroid metabolism have been described in Figure 1. The major pathways of metabolism of iodine that occur outside of the thyroid gland involve the catabolism of T4 and T3, and include deiodination reactions, ether bond cleavage of thyronine, oxidative deamination and decarboxylation of the side chain of thyronine, and conjugation of the phenolic hydroxyl group on thyronine with glucuronic acid and sulfate, Figure 3.

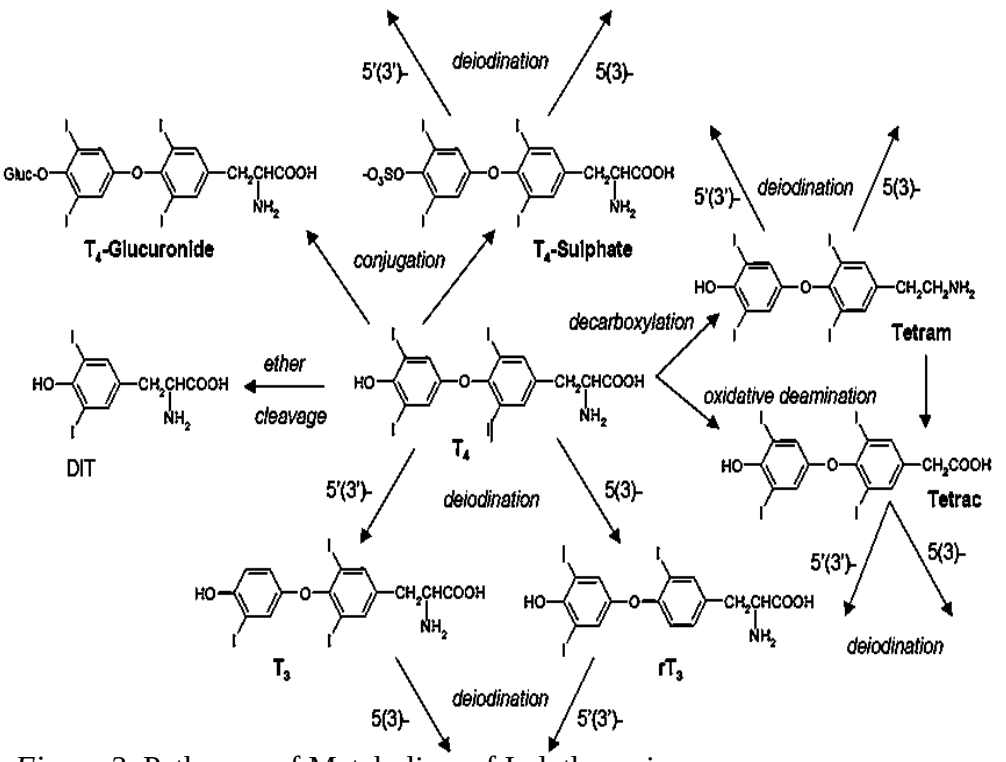


Figure 3: Pathways of Metabolism of Iodothyronines.

Source: ATSDR, 2004

Deiodination products formed in peripheral tissues are depicted in Figure 4.

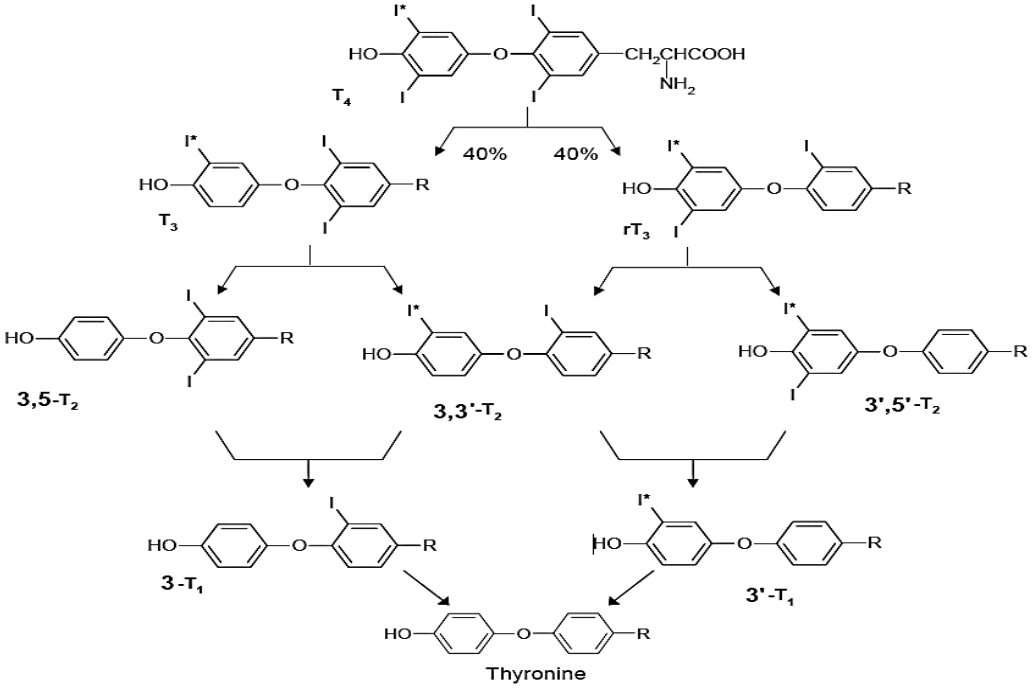


Figure 4: Major Deiodination of Pathways of Thyroid Hormones in Peripheral Tissues.

Source: ATSDR, 2004

The liver and kidney are thought to be major sites of production of T3 in the circulation; however, local tissue production of T3 from T4 is thought to be the predominant source of T3 in the brain and pituitary. Iodothyronine deiodinases also catalyze the inactivation of T4 and T3. The activities of deiodinases are under feedback control, mediated by T3, T4, and reverseT3 (rT3), an inactive deiodination product of T4 (Peeters et al., 2001). Deiodination of T4 and T3 also functions to deactivate the thyroid hormones. Iodide released from the deiodination reactions is either taken up by the thyroid gland or excreted in urine. Deiodination is catalyzed by selenium-dependent deiodinase enzymes (selenodiodinases). Oxidative deamination and decarboxylation of the alanine side chain of the iodothyronines represents approximately 2 and 14% of total of T4 and T3 turnover, respectively. Enzymes that catalyze these reactions have not been well characterized.

Urinary excretion normally accounts for >97% of the elimination of absorbed iodine, while fecal excretion accounts for approximately 1-2% (Hays, 2001). The whole-body elimination half-time of absorbed iodine has been estimated to be approximately 31 dys in healthy adult males (Hays, 2001), however, there appears to be considerable inter-individual variability in the half-time. Simon et al., (2002) estimated a transfer coefficient for ^{131}I from intake to breast milk (ratio of steady-state ^{131}I concentration in breast milk to ^{131}I intake rate) to be approximately 0.12 dy/L milk ("1.5 SD). The fraction of the absorbed iodide dose excreted in breast milk varies with functional status of the thyroid gland and with iodine intake. A larger fraction of the absorbed dose is excreted in breast milk in the hypothyroid state compared to the hyperthyroid state. In the hypothyroid state, uptake of absorbed iodide into the

thyroid and incorporation into iodothyronines is depressed, resulting in greater availability of the absorbed iodide for distribution to the mammary gland and breast milk. Several examples of this have been reported in the clinical case literature (Zamrazil, 2009; Vandecasteel et al., 2000).

Iodide is secreted in saliva in humans, salivary secretion of iodide may be an important pathway for recycling of iodine (Mandel & Mandel, 2003). The quantitative contribution of the saliva pathway to excretion of iodine has not been reported, and is probably minimal, given the relatively small rate of production of saliva under normal circumstances, most of which is ingested. Appreciable amounts of iodide can be excreted in sweat, under conditions of strenuous physical activity (Mao et al., 2001). Iodide appears to be excreted into the intestine by a mechanism other than biliary secretion of iodothyronine (and metabolic conjugates). Evidence in support of this comes from observations of radioactivity in the colon of patients who have no functioning iodothyronine production and who received doses of radioiodine.

Absorption and Transport of Iodine

The mechanism(s) by which iodide is absorbed from the gastrointestinal tract appears to occur primarily in the small intestine in humans and that the stomach may play a minor role in iodide absorption. The mechanisms by which iodide is transported across the intestinal epithelium are not known. Specifically, Uptake of iodide into the thyroid is facilitated by a membrane carrier in the basolateral membrane of the thyroid follicle cell (Shen et al., 2001).

Iodothyronine Transport

Uptake of T4 and T3 into tissues occurs by a saturable, energy-dependent carrier transport system. Evidence for active transport derives from a variety of observations. The rate of uptake of T3 into the perfused rat liver is proportional to the concentration of free T3 in the perfusate and is not related to the total concentration or bound concentration. The free cytosolic concentration of T3 in the in vivo rat liver and heart muscle exceeds that of the simultaneous free concentration in plasma, suggesting uptake of T3 into these tissues against a chemical gradient for T3 uptake into confluent cultures of human or rat hepatoma cells is saturable, stereoselective for the active L enantiomer, temperature dependent, and inhibited by metabolic and membrane transport inhibitors, including phloretin. Saturable, stereoselective, temperature-dependent, and energy-dependent uptake of T3 and T4 has also been observed in cultures of human fibroblasts and of T3 in in vitro preparations of rat skeletal muscle (ATSDR, 2004).

Iodination in the Thyroid Gland

Iodination of thyroglobulin is catalyzed by thyroid peroxidase, a hemoprotein in the apical (luminal) membrane of thyroid follicle cells (Dunn & Dunn, 2001). Thyroid peroxidase catalyzes both the iodination of tyrosine residues in thyroglobulin and the coupling of the iodinated residues to form the thyroid hormones, T4 and T3, and diiodotyrosine. The iodination reaction involves the oxidation of iodide (I^-) to a reactive species having a sufficiently high oxidation potential to iodinate the aromatic ring of tyrosine. The oxidizing agent in the reaction is hydrogen peroxide, which is generated at the apical membrane of follicle cells by an NADPH oxidase. Although the exact

mechanism of the iodination reaction is not completely understood, three species are suspected as being candidates for the reactive iodinating species: a free radical (I), iodinium (I^+), or an enzyme-bound hypoiodite (EOI^-).

Deiodination of Iodothyrones in Peripheral Tissues

Deiodination serves as an important mechanism for the production of extrathyroidal T3 and as the deactivation of the thyroid hormones, T4 and T3. The deiodination reactions are catalyzed by selenium-dependent deiodinase enzymes (selenodeiodinases). Three selenodeiodinases have been described that differ in substrate preference, reaction products, response to inhibitors (propylthiouracil, gold), and response to T3. Full activity of each enzyme requires selenocysteine in the amino acid sequence of the active site, which is the basis for deiodination activity being responsive to nutritional selenium status (ATSDR, 2004).

Urinary Excretion of Iodide

Urinary excretion normally accounts for more than 97% of the elimination of absorbed iodine. The renal plasma clearance of iodine has been measured in human subjects during continuous intravenous infusions of radioiodide. Under these conditions, only a negligible amount of radioiodine in the plasma was associated with protein and more than 98% was ultrafilterable; thus, the renal clearance of radioiodine can be assumed to reflect that of radioiodide.

Under steady-state conditions with respect to the serum radioiodine concentration, the renal plasma clearance of radioiodine was approximately 30% of the glomerular filtration rate, suggesting that filtered iodide is reabsorbed in the renal tubule. The mechanism of renal tubular reabsorption of

iodide has not been elucidated, although studies to examine mechanisms have been largely limited to clearance studies. NIS mRNA is expressed in human kidney and NIS immunoreactivity has been observed in the human kidney proximal and distal tubules; however, its role in iodine transport in the kidney has not been elucidated (Spitzweg et al., 2001). In humans, iodide clearance as a fraction of the glomerular filtration rate (CI/GFR) increases in response to an acute increase in GFR and decreases in response to an acute decrease in GFR.

Mechanisms of Toxicity

The mechanism by which excess iodide produces hypothyroidism, is not completely understood. Iodide excess inhibits the iodination of thyroglobulin in the thyroid gland and inhibits the release of T4 and T3 from the gland (Medani et al., 2013). Both effects could contribute to stimulation of release of TSH from the pituitary gland and to the increase in serum concentration of TSH and hypertrophy of the thyroid gland that has been shown to accompany iodide-induced thyroid gland suppression.

Toxicities mediated through the neuroendocrine axis

Iodine is an endocrine disruptor in that the principal direct effects of excessive iodine ingestion are on the thyroid gland and on the regulation of thyroid hormone production and secretion. The effects of iodine on the thyroid gland include hypothyroidism, hyperthyroidism, and thyroiditis. The above three types of effects can occur in children and adults, and in infants exposed in utero or during lactation. Adverse effects on the pituitary and adrenal glands derive secondarily from disorders of the thyroid gland. A wide variety of effects on other organ systems can result from disorders of the thyroid gland, including disturbances of the skin, cardiovascular system, pulmonary system,

kidneys, gastrointestinal tract, liver, blood, neuromuscular system, central nervous system, skeleton, male and female reproductive systems, and numerous endocrine organs, including the pituitary and adrenal glands (Braverman & Utiger, 2000).

Children's Susceptibility

Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight, the gastrointestinal absorption of lead is greatest in infants and young children.

At various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults, and sometimes unique enzymes may exist at particular developmental stages. Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in newborns who all have a low glomerular filtration rate and have not developed efficient tubular secretion and resorption capacities. Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer remaining lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

Nutritional factors can affect the toxicokinetics of iodine in children and adults. The most important factor is dietary iodine. Chronic iodine deficiency triggers homeostatic mechanisms to increase iodide uptake into the thyroid gland in order to sustain adequate thyroid hormone levels to regulate metabolism.

Another nutritional factor that could potentially affect iodine biokinetics in infants and children is selenium deficiency. Selenium is a cofactor in the iodothyronine deiodinases that are important for the synthesis of the thyroid hormone, T3, in extrathyroidal tissues. Iodine deficiency, in conjunction with selenium deficiency, has been associated with goiter and cretinism, a developmental impairment related to prenatal hypothyroidism.

Interactions of Iodide with other Chemicals (Goitrogens and Anti-Goitrogens)

These include thioureylenes and thionamides, analine derivatives, substituted phonols, hydroxypyridines, perchlorate and related complex anions. They also include thiocyanate, microsomal enzyme inducers, bolychlorinated biphenols (PCBs), selenium, amiodarone, lithium, propranol, dexamethasome and iodinated drugs (ATSDR, 2006)

Iodine Deficiency and Iodine Deficiency Disorders (IDD)

The main factor responsible for iodine deficiency is a low dietary supply of iodine (Hörmann, 2005). It occurs in populations living in areas where the soil has low iodine content as a result of past glaciation or the repeated leaching effects of snow, water and heavy rainfall. Crops grown in this soil, therefore, do not provide adequate amounts of iodine when consumed.

Iodine is present in the body in minute amounts, mainly in the thyroid gland. Its main role is in the synthesis of thyroid hormones. When iodine requirements are not met, thyroid hormone synthesis is impaired, resulting in hypothyroidism and a series of functional and developmental abnormalities grouped under the heading of "Iodine Deficiency Disorders (IDD)", this term emphasis that the problem extends beyond simply goiter and cretinism.

Iodine is an essential micronutrient. The nutritional requirement for iodine (under review by WHO) is currently in the range of 0.10 to 0.14 mg/dy for adults. The committee set a provisional maximum tolerable daily intake of 1mg iodine/day (0.017 mg/kgbw) from dietary sources. This level may cause adverse effects for the thyroid patients or those particularly sensitive to iodine.

Iodine deficiency is a major Public Health problem for populations throughout the world, particularly for pregnant women and young children. They are a threat to the social and economic development of countries. The most devastating outcomes of iodine deficiency are increased perinatal mortality and mental retardation. It affects the protective and the reproductive status of a population.

Iodine deficiency can be as a result of a natural ecological phenomenon that occurs in many parts of the world due to erosion of soils in riverine areas; and loss of vegetation from clearing for agricultural production, overgrazing by livestock, and tree-cutting for firewood results in a continued and increasing loss of iodine from the soil. Foods grown locally in these areas lack iodine, and hence consumption of such foods and food products does not supply the body with the required amount of iodine.

The most critical period is from the second trimester of pregnancy to the third year after birth (Delange, 2000). Normal levels of thyroid hormones are required for optimal development of the brain. In areas of iodine deficiency, where thyroid hormone levels are low (hypothyroidism), brain development is impaired resulting in cretinism, but of much greater public health importance are the more subtle degrees of brain damage and reduced cognitive capacity which affects the entire population.

A complete list of susceptible groups is presented in Table 4.

Table 4: The Spectrum of Iodine Deficiency Disorders

Physiological Groups	Health Consequences of Iodine Deficiency
All ages	Goitre
	Hypothyroidism
	Increased susceptibility to nuclear radiation
Fetus	Spontaneous abortion
	Stillbirth, deaf mutism
	Congenital anomalies
	Perinatal mortality
Neonate	Endemic cretinism including mental deficiency with a mixture of mutism, increased susceptibility of thyroid gland to nuclear radiation
	spastic diplegia, squint, hypothyroidism and short stature
	Infant mortality, neo-natal (goiter, neo-natal hyperthyroidism
Child and adolescent	Impaired mental function, goitre
	Delayed physical development
	Iodine-induced hyperthyroidism (IIH)
Adults	Impaired mental function, goitre
	Iodine-induced hyperthyroidism (IIH)

Source: WHO A Guide for Programme Managers 3rd ed. 2007.

Acute intakes of 700 µg/dy or 10 µg/kg/dy had no detectable effect on thyroid status in healthy individuals. One study found no evidence for disturbances in thyroid hormone status in healthy adults who received doses of 300 µg/kg/dy (approximately 20 mg/dy) for 14 dys. This suggests that, at least under certain conditions, exposure levels >10-24 µg/kg/dy may be tolerated in some people. Brief summaries of the relevant studies that provide information on oral exposures to iodine that suppress the thyroid gland are provided below.

Zhao et al., (2000), compared the prevalence of thyroid enlargement among children 5-15 yrs of age to drinking water and urinary iodine levels in residents of 65 townships in Jiangsu Province, China. This area had a high prevalence of childhood goiter, although urinary iodide measurements sµggested dietary iodine sufficiency. Urinary iodine measurements were obtained for adults who resided in the same townships as the children. The prevalences of goiter and abnormal thyroid volume (not defined in the report) increased with increasing urine iodine concentration. The prevalences of goiter increased from 15% (802 µg I/L urine) to 38% (1,961 µg I/L urine). The prevalences of abnormal thyroid volume increased from 5-17% over this same range of urinary iodine concentrations. Assuming an adult urine volume of 1.4 L/day and an adult body weight of 60 kg, the observed range of urinary iodide concentrations in adults (520-1,961 µg I/L) corresponded to approximate intakes of 730-2,750 µg/dy (12-46 µg/kg/dy).

A survey of a group of Peace Corps volunteers revealed a high prevalence of goiter among volunteers who drank water from iodine filters. Of 96 volunteers surveyed, 44 (46%) had enlarged thyroid glands, 33 (34%) had elevated serum TSH concentrations (4.2 mU/L), and 4 (4%) had

depressed serum TSH concentrations (0.4 mU/L). The mean iodide concentration in filtered drinking water was 10 mg I/L, which corresponded to a daily intake of iodide from drinking water of 50-90 mg I/dy (0.7-1.3 mg/kg/dy, based on a reported daily water consumption of 5-9 L/dy). This estimate was consistent with measured mean urinary iodide concentration of 11 mg/L, which corresponds to approximately 55-99 mg I/dy excreted or ingested, assuming daily urine volumes similar to water consumption. When the excess iodine was removed from the drinking water, all measures of thyroid function returned to normal (Pearce et al., 2002).

A study of iodine supplementation for treatment of endemic goiter related to iodine deficiency provides additional evidence that increases in iodine intake can induce thyroid dysfunction, including thyroid autoimmunity. Otherwise healthy adults who had goiter but no evidence of clinical hypothyroidism or hyperthyroidism or antithyroid antibodies received either a placebo (16 females, 15 males) or 200 μg I/dy (3 μg/kg/dy total intake) (16 females, 15 males) as potassium iodide for 12 mths. A significant decrease in thyroid volume occurred in the treated group relative to the control group. Three subjects in the treatment group (9.7%, two females and one male) developed elevated levels of thyroglobulin and thyroid microsomal antibodies compared to none in the control group. Two of these subjects developed hypothyroidism and one subject developed hyperthyroidism; all three subjects reverted to normal thyroid hormone status when the iodide supplementation was discontinued.

Iodised oil has been used to supplement intakes in populations that are iodine deficient in areas where supplementation with iodised table salt or

drinking water is not practical. Iodised oil (ethiodiol) consists of a mixture of covalently iodinated fatty acids of poppy seed oil; the iodine content is approximately 38% by weight. Iodine in iodised oil is taken up in adipose tissue and has a much longer retention time in the body than iodide salts; thus, epidemiological studies of iodised oil cannot be directly compared to those of iodide salt. Nevertheless, the studies provide some useful information on oral exposures to iodine that are tolerated during pregnancy without apparent adverse consequences to the fetal or neonatal thyroid.

A large multinational epidemiological study was conducted in Africa to evaluate the effectiveness and possible adverse consequences of the introduction of iodised salt into diets of populations residing in iodine-deficient and endemic goiter regions of Africa. In each study area, urine and table salt were collected from a group of 100-400 randomly-selected children, ages 6-14 yrs. Health care facilities were surveyed for information on thyroid disease in each area. In Zimbabwe, the incidence of hyperthyroidism increased by a factor of 2.6 within 18 months after the widespread introduction of iodised salt into the diet (from 2.8 in 100,000 to 7.4 in 100,000). Females accounted for 90% of the cases, with the highest incidence in the age group 60-69 yrs. The most common disorders were toxic nodular goiter (58%) and Graves' disease (27%), urinary iodide concentration in children increased by a factor of 5-10 over this time period. Urine samples were reported as "casual samples" and, thus, there is a large uncertainty in translating the concentrations into intakes. Median urine iodide concentrations ranged from 290-560 µg/L. Reported estimates of iodide intake from salt and seafood were 500 µg/dy (7.1 µg/kg/dy) and 15-100 µg/dy (0.2-1.4 µg/kg/dy), respectively.

Increased numbers of cases of thyrotoxicosis along with an increase in urinary iodide levels (from 16-240 μg/L) occurred after iodized salt was introduced into the diet of an iodine-deficient population in the Kivu region of Zaire.

In an experimental study, adults with goiter who lived in an iodine-deficient region of Sudan received a single oral dose of 200, 400, or 800 mg iodine (3-11 mg/kg/dy) as iodine oil (37-41 subjects per dose group) and their thyroid status was evaluated for a period of 12 mths (Dunn, 2001) Approximately, half of the subjects were clinically hypothyroid with serum T4 concentrations <50 nmol/L and TSH concentrations >4 mU/L. One week after the iodine oil was administered, there was a dose-related increase in the incidence of serum TSH concentrations; 1 in 41 (2.5%) in the low-dose group, 3 in 37 (8.1%) in the middle-dose group, and 10 in 39 (25.6%) in the high-dose group, although the number of subjects exceeding 4 mU/L was not dose-related. One subject in the low-dose group and three subjects in the high-dose group became hyperthyroid during the observation period. One of the high dose subjects remained hyperthyroid 1 year after the dose of iodine oil. There is global inadequate iodine nutrition of 30.6% covering a population of 1901 million, with a 70% access to iodated salt by household. Specifically, Western Pacific with accessibility of 89.5% has 21.2% of inadequate iodine nutrition whilst Europe with 49.2% accessibility has 52.0% inadequate iodine nutrition. The reason for the inadequacy includes poor implementation of the programme, iodine losses during cooking, increased goitrogens in environment, diet and water (Kotwal et al., 2007). The importance of IDD through its effects on the developing brain, has condemned millions of people to a life of few prospects and continued underdevelopment. On a worldwide

basis, iodine deficiency is the single most important preventable cause of brain damage.

People living in areas affected by severe iodine deficiency may have an IQ of up to 13.5 points below that of those from comparable communities in areas where there is no iodine deficiency.This mental deficiency has an immediate effect on child learning capacity, women's health, the quality of life in communities, and economic productivity. (WHO/UNICEF/ICCIDD, 2007).

On the other hand, IDDs are among the easiest and least expensive of all nutrient disorders to prevent. The addition of a small, constant amount of iodine to the salt that people consume daily is all that is needed. The elimination of IDD is a critical development issue, and should be given the highest priority by governments and international agencies (WHO/UNICEF/ICCIDD, 2001).

While IDD affects the entire population, a school-based sampling method is recommended for urinary iodine (UI) and Total Goitre Prevalence (TGP) as the most efficient and practical approach to monitor IDD as this group is usually easily accessible and can be used as a proxy for the general population (WHO/UNICEF/ICCIDD, 2001). School-age refers to children aged 6-12 yrs. Iodine deficiency is considered to be a public health problem in populations of school-age children where the median UI < 100 μg/L or goitre prevalence is above 5% (WHO/UNICEF/ICCIDD, 2001). The most visible disorders of iodine deficiency are goiter and cretinism, and the next section discusses these disorders.

Goiter

It is the swelling of the neck or larynx resulting from enlargement of the thyroid glands (thyromegaly), Plate 1-3.

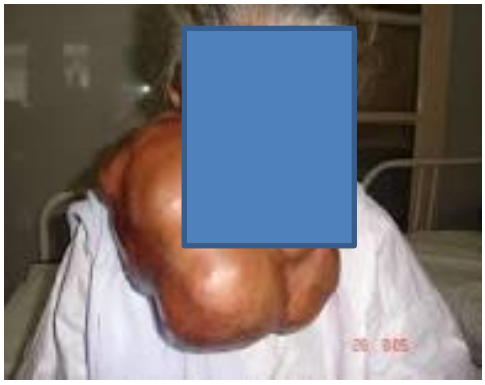


Plate 1: A goitrous male

Plate 2: Agoitrous female

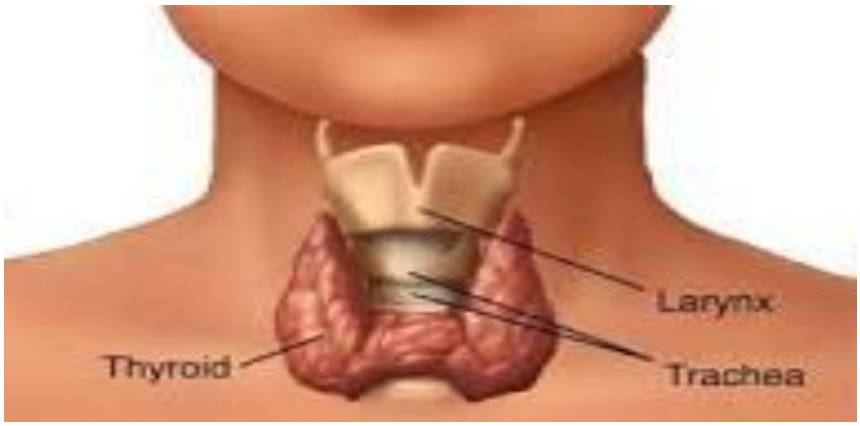


Plate 3: An illustration of the thyroid gland, trachea and the larynx of a normal person

World wide over 90% cases of goiter are caused by iodine deficiency (Hormann, 2005). Goiter leads to the enlargement of the thyroid gland to compensate for the secretion of the hormone thyroxin. The change in size and quality of the gland does not seem to have noticeable effects on the person, other than in physical appearance of the bulging out of the neck region of humans. Goiter also occurs in animal which has a thyroid gland, for example, pig, sheep and fish.

Goiter associated with hypothyroidism or hyperthyroidism, may be present with symptoms of the underlying disorder. For instance in hyperthyroidism, the most common symptoms include tachycardia, palpitations, nervousness, tremor, increased blood pressure, and heat intolerance. Clinical manifestations are often related to hypermetabolism (increased metabolism), excessive thyroid hormone, an increase in oxygen consumption, metabolic changes in protein metabolism, immunologic stimulation of diffuse goiter, and ocular changes (exophthalmos) (Porth et al., 2011). Hypothyroid individuals may have weight gain despite poor appetite, cold intolerance, constipation and lethargy. Regarding morphology, goiters may be classified either as the growth pattern or as the size of the growth. For growth pattern, goiter is either uninodular, multinodular or diffuse goiter. Uninodular goiter can be either inactive or a toxic nodule just as multinodular. However, in diffuse goiter, the whole gland appears to be enlarged. By size it is classified as Class I, Class II, and Class III.

Class I type cannot be seen in normal posture of the head, it is only found by palpation. Class II goiter is palpable and can be easily seen whilst the Class III type is very large and is retrosternal; pressure results in compression marks. The causes of goiter are due to iodine deficiency, selenium deficiency and Hashimoto's thyroiditis (in countries that use iodised salt). It can also result from cyanide poisoning; this is particularly common in tropical countries where people eat the cyanide rich cassava root as the staple food (ATSDR, 2006). Other causes include congenital hypothyroidism, goitrogen ingestion, adverse drµg reactions, pituitary disease and Grave's disease (Basedon syndrome). It also includes thyroiditis, thyroid cancer, benign

thyroid neoplasms, and thyroid hormone insensitivity. The rest are sarcoidosis, hydatidifarm mote, cysts, acromegaly, and pendred syndrome.

Cretinism

Another disorder involves tissue atrophy and decrease in thyroid secretions. This is known as cretinism in the young, myxydemia in the adult. In the hypothyroid state the rate of secretion of the pituitary growth hormone is lessened; this explains the lack of growth in the cretin. A cretin is defined as the person who is physically deformed and has learning difficulties because of congenital thyroid deficiency. A person suffering from cretinism, is a stupid, obtuse, and mentally defective person. Cretinism is usually a congenital abnormal condition marked by physical stunting and mental retardation and caused by severe thyroid deficiency. This is also called infantile myxedemia, mostly caused by genetic mutations. These involve anomalies in the production of enzymes that are needed for the thyroid hormone synthesis. Some of the underlying causes of cretinism are: hereditary in origin, missing or misplaced thyroid gland, maternal iodine levels and maternal thyroid condition and medications, and dysfunction of the pituitary gland/hypothalamus. A small percentage of cretinism patients have inherited anomalous genes that cause the thyroid gland to produce less thyroid hormone (hypothyroidism). In most babies born with cretinism, the thyroid is either absent or underdeveloped or located differently such as under the tongue or side of the neck instead of the centre and front f the neck, and near the top of the windpipe. In some cases, the thyroid is smaller than the normal size. Such abnormalities result in either less or no thyroid hormone production. This kind of defect is not inherited. When there is maternal iodine deficiency, there will

be insufficient amount of iodine for the production of thyroid hormones. The thyroid of the fetus is also affected by too much iodine during pregnancy (Hormann, 2005; Laurberg et al., 2000). There are cases wherein the mother of the fetus is diagnosed with a thyroid problem. The treatment which involves antithyroid glands also contributes to the occurrence of cretinism (Laurberg et al., 2000). This occurs in 5% of cretinism patients. The thyroid glands is not stimulated by the pituitary gland, and the hypothalamic causes of cretinism include ischemic damage, congenital detects and tumors.

Initiatives by Countries to Eliminate IDD

Today goitre and cretinism are still observed among adults and children. Its occurrence in some parts of the world has been reduced because of the application of scientific knowledge concerning its prevention and cure. Hence the widespread use of iodised salt led to a reduction of IDD. In the US for e.g., Michigan school children's goiter incidence was 47% in 1921, and a little over 1% in 1951 in Ohio, 32% in 1925 and 4% in 1954. The USA Food and Drug Administration recommended 150 μg (0.15mg) of iodine per day for both men and women.

Canada has also handled the situation by adopting in 1949 compulsory iodisation of table salt. Then most countries like Guatemala, Colombia, Switzerland and Australia have also made salt iodization mandatory. Brazil detected IDD in the 1950s, about 20% of the population had goitre. Brazilian consume about 12 g of table salt per dy which is strongly linked to the most common cause of death, that is, vascular disorders, the maximum allowable fortification level of 60 mg/kg would lead to a daily intake of 0.72 mg of

iodine, many times above the recommended 0.13 mg adults and 0.20 mg pregnancy/breast feeding consumption.

A report by UNICEF and Iodine Global Network (IGN) about Latin America indicates adequate iodine intake exemplified by Sri Lanka. Sri Lanka has created a successful salt iodization programme that has reduced goiter from 20.9% to just 3.8% ten years later. In Northern Ireland pregnant women commonly suffer iodine deficiency, but offspring have adequate iodine levels. Iodine deficiency is still a problem in the northern provinces of Thailand. The department of medical sciences has suggested a control and prevention plan which includes more research and monitoring of the deficiency. In China, UNICEF has emphasized the importance of maintaining universal availability of iodised salt to protect China's pregnant women and children against IDD in spite of their successes.

Global Iodine Incidence

Goiter had a global character as shown in Table 5.

Table 5: Global Character of Goiter and its Prevalence

WHO Region	Total Goiter Prevalence %			PercentageChange Between 1993 and 2004
	1993	1997	2004	
Africa	15.6	20.0	28.3	+81.4
America	08.7	05.0	04.7	46.0
Southeast Asia	13.0	12.0	15.4	+18.5
Europe	11.4	15.0	20.6	+80.7
Eastern Mediterranean	22.9	32.0	37.3	+62.9
Western Pacific	09.0	08.0	06.1	32.2
Total	12.0	13.0	15.8	+31.7

Source: WHO A Guide for Programme Managers 2nd ed; Kotwal et al., 2007

Between 1993 and 2004, IDD generally increased globally (31.7%). Africa registered the highest percentage followed by Europe with percentage of 80.7%. However, America and Western Pacific recorded a decline of 46% and 32.2% respectively, Table 5. This observation means that strategies adopted for the control of goiter and other IDDs are not achieving their aim (Kotwal et al., 2007). The TGP increase of 31.7% worldwide has masked a decrease in two regions of 46% in the Americas and 32.2% in the Western Pacific (WHO A Guide for Programme Managers, 2004).

Another publication (WHO/UNICEF/ICCIDD, 2004) indicates that there is an overall increase of 70% of households with access to iodited salt worldwide. Americas and the Western Pacific have the highest access of 89.8% and 89.0% respectively, whilst Europe and Eastern Mediterranean recorded the lowest. African and South East Asia were intermediate, Table 6.

Table 6:Proportion of Population and Number of Individuals in the General Population (all age groups) with Insufficient Iodine Intake by WHO Regions during the period between1994 and 2006,a,b and Proportion of Households using 'c' iodised salt

WHO regions	Inadequate Iodine proportion (%) a	Nutrition total number (million) b	% household with access to iodized salt c
Africa	41.5	312.9	66.6
Americas	11.0	98.6	89.8
South-East Asia	30.0	503.6	61.0
Europe	52.0	459.7	49.2
Eastern Mediterranean	47.2	259.3	47.3
Western pacific	21.2	374.7	89.6
Total	30.6	1900.9	70.0

Source: Iodine Status Worldwide, 2004

a. Based on surveys from 130 countries made available to WHO and carried out between January 1994 and December 2006.
b. Country data on proportion of households using iodized salt based on UNICEF global database
c. UN population division. World population prospects: the 2004 revision. New York, United Nations, 2005.

Monitoring and Evaluating IDD Control Programmes

Salt iodisation programmes, require an effective system for monitoring and evaluation. The challenge is to apply the IDD indicators using valid and reliable methods while keeping costs to a minimum. In some countries there is still inadequate information on IDD, and programmes have not yet been implemented. The various indicators of impact which are used in monitoring and evaluating IDD control programmes are divided into three main groups: urinary iodine (UI); thyroid size by palpation and/or by ultrasonography; and the blood constituents: TSH or thyrotropin, and thyroglobulin.

Urinary Iodine

Urinary Iodine (UI) is a more sensitive indicator to recent changes in iodine intake. It is now recommended over TGP (WHO/UNICEF/ICCIDD, 2001). Most countries have started to implement IDD control programmes, and a growing number of countries are consequently monitoring iodine status using UI. Since most countries have now started to implement IDD control programmes, urinary iodine rather than thyroid size is emphasized as the principal indicator of impact. Thyroid size is more useful in baseline assessments of the severity of IDD, and also has a role in the assessment of the long-term impact of control programmes. Most iodine absorbed in the body eventually appears in the urine. Therefore, urinary iodine excretion is a good marker of very recent dietary iodine intake. In individuals, urinary iodine excretion can vary somewhat from day to day and even within a given day. However, this variation tends to even out among populations. Studies have convincingly demonstrated that a profile of iodine concentrations in morning or other casual urine specimens (child or adult) provides an adequate

assessment of a population's iodine nutrition, provided a sufficient number of specimens are collected. Round the clock urine samples are difficult to obtain and are not necessary. Relating urinary iodine to creatinine, as has been done in the past, is cumbersome, expensive, and unnecessary. Indeed, urinary iodine/ creatinine ratios are unreliable, particularly when protein intake and consequently creatinine excretion is low (WHO A Guide for Programme Managers, 2004). Acceptance of this indicator is very high, and casual urine specimens are easy to obtain. Urinary iodine assay methods are not difficult to learn or use, but meticulous attention is required to avoid contamination with iodine at all stages. Special laboratory areas, glassware, and reagents should be set aside solely for this determination.

In general, only small amounts (0.5-1.0 mL) of urine are required, although the exact volume depends on the method. Some urine should also be kept in reserve for replicate testing or for external quality control. Samples are collected in small cups and transferred to tubes, which should be tightly sealed with screw tops. They do not require refrigeration, addition of preservative, or immediate determination in most methods. They can be kept in the laboratory for months or more, preferably in a refrigerator to avoid unpleasant odour.

Evaporation should be avoided, because this process artificially increases the concentration. Samples may safely be frozen and refrozen, but must be completely defrosted before aliquots are taken for analysis.

Analytical Methods for Determining Urinary Iodine Concentrations

Many analytical techniques exist, varying from very precise measurement with highly sophisticated instruments, to semi-quantitative 'low tech' methods that can be used in regional, country, or local laboratories. The

method that is mostly used is the Sandell-Kolthoff method, it depends on iodine's role as a catalyst in the reduction of ceric ammonium sulfate (yellow colour) to the cerous form (colourless) in the presence of arsenious acid (the Sandell-Kolthoff reaction). A digestion or other purification step using ammonium persulfate or chloric acid is necessary before carrying out this reaction, to rid the urine of interfering contaminants (WHO A Guide for Programme Managers, 2007). A brief description of the varieties of Sandell-Kolthoff methods are presented in the next section.

The Cerric Ammonium Sulfate Method (Method A)

Small samples of urine (0.25-0.5 mL) are digested with ammonium persulfate at 90-110 °C; arsenious acid and ceric ammonium sulfate are then added. The decrease in yellow colour over a fixed time period is then measured by a spectrophotometer and plotted against a standard curve constructed with known amounts of iodine (Medani et al., 2013). This method requires a heating block and a spectrophotometer, which are both inexpensive instruments. About 100-150 samples can be run in a day by one experienced technician.

The Chloric Acid Method (Method B)

Chloric acid can be substituted for ammonium persulfate in the digestion step, and the colorimetric determination carried out as for method A disadvantage is the safety concern, because the chemical mixture can be explosive if residues dry in ventilating systems. Handling these chemicals in a fume cupboard and using a chloric acid trap when performing sample digestion is strongly recommended. Other methods include modification of method B, which uses the redox indicator ferroin and a stopwatch instead of a

spectrophotometer to measure colour change. Urine is digested with chloric acid and colour changes in batches of samples measured relative to standards of known iodine content. This places samples in categories (for example, 50, 50-100, 100–200 μg/L, etc.) that can be adjusted to desired levels. This method is currently being adapted to ammonium persulfate digestion. Another, semi-quantitative method is based on the iodide-catalysed oxidation of 3,3',5,5'-tetramethylbenzidine by peracetic acid/H_2O_2 to yield coloured products that are recognized on a colour strip indicating three ranges: <100, 100-300, and >300 μg/L. Interfering substances are removed by pre-packed columns with activated charcoal. Analyses must be run within two hours, and the procedure requires the manufacturer's pre-packed columns. In still another method, samples are digested with ammonium persulfate on microplates enclosed in specially designed sealed cassettes and heated to 110°C (Ohasi et al., 2000). Samples are then transferred to another microplate and the ceric ammonium sulfate reduction reaction carried out and read on a microplate reader. Field tests are promising: up to 400 urine samples can be analysed in one day, depending on manufacturers' supplies.

Choice of Method of Analysis

Criteria for assessing urinary iodine methods are reliability, speed, technical demands, complexity of instrumentation, independence from sole source suppliers, availability of high quality reagents, safety, and cost. The choice among the above and other methods depends on local needs and resources. Large central laboratories processing many samples may prefer 'high-tech' methods, while smaller operations closer to the field may find the simplest methods more practical. Due to the potential hazards of Chloric acid,

Method A that uses Ammonium persulfate is currently recommended. It can adequately replace the chloric acid method, since the main difference is the substitution of ammonium persulfate for chloric acid in the digestion step.

The other methods described above show promise but are not yet fully tested (WHO A Guide for Programme Managers, 2007). Most of the other methods perform reliably, although some of the newer ones need further testing as of this date. With appropriate dilutions, they can be extended upward to examine whatever range is desired. The coefficient of variation is generally under 10% for all methods. Proper training is necessary but not complicated. Since casual specimens are used, it is desirable to measure a sufficient number from a given population to allow for varying degrees of subject hydration and other biological variations among individuals, as well as to obtain a reasonably narrow confidence interval. In general, 30 urine determinations from a defined sampling group are sufficient.

The Medium Urinary Iodine Concentration

Simple modern methods make it feasible to process large numbers of samples at a low cost and to characterize the distribution according to different cut-off points and intervals. The cut-off points proposed for classifying iodine nutrition into different degrees of public health significance are shown in Tables 7 and 8.

Table 7: Epidemiological Criteria for Assessing Iodine Nutrition based on Median Urinary Iodine Concentrations of School-Age Children (≥6 yrs)

Median urinary iodine (µg/L)	Iodine intake	Iodine status
< 20	Insufficient	Severe iodine deficiency
20–49	Insufficient	Moderate iodine deficiency
50–99	Insufficient	Mild iodine deficiency
100–199	Adequate	Adequate iodine nutrition
200–299	Above requirements	Likely to provide adequate intake for pregnant/lactatingwomen, but may pose a slight risk of more than adequate intake in the overall population
≥300	Excessive	Risk of adverse health consequences (iodine-induced hyperthyroidism, autoimmune thyroid diseases)

Source: WHO A Guide for Programme Managers 3rd ed. 2007

Table 7 applies to adults, and not to pregnant and lactating women.

Epidemiological Criteria for Assessing Iodine Nutrition based on the Median or Range in Urinary Iodine Concentrations of Pregnant women was also sampled for urinary iodine, thus establishing a normal range, Table 8

Table 8: Epidemiological Criteria for Assessing Iodine Nutrition based on the Median or Range in Urinary Iodine Concentrations of Pregnant women

Population group	Median urinary iodine concentration (µg/L)	Iodine intake
Pregnant women	< 150	Insufficient
	150-249	Adequate
	250-499	Above requirements
	≥ 500	Excessive

Source: WHO A Guide for Programme Managers 3rd ed. 2007

For lactating women and children <2 yrs of age a median urinary iodine concentration of 100 µg/L can be used to define adequate iodine intake, but no other categories of iodine intake are defined. Although lactating women have the same requirement as pregnant women, the median urinary iodine is lower because iodine is excreted in breast milk. The term "excessive" means in excess of the amount required to prevent and control iodine deficiency.

The median value for the sampled population is the most commonly assessed indicator. Urinary iodine values from populations are usually not normally distributed. Therefore, the median rather than the mean is used as the measure of central tendency. Likewise, percentiles rather than standard deviations should be used as measures of spread. Frequency distribution curves can also be very useful for full interpretation, particularly if there is salt iodine level data available for the same population. In children and non-pregnant women, median urinary iodine concentrations of between 100 and 99 µg/L define a population which has no iodine deficiency. In addition, not more than 20% of samples should be <50 µg/L. In non-pregnant, non-lactating women, a urinary iodine concentration of 100 µg/L corresponds roughly to a daily iodine intake of about 150 µg under steady-state conditions. During pregnancy, median urinary iodine concentrations of between 150 and 299 µg/L define a population which has no iodine deficiency (WHO Technical Consultation, 2007). Establishing the ideal range of values for urinary iodine is difficult. By definition, when the median is 100 µg/L, at least 50% of the samples will be lower than 100 µg/L. Historically, schoolchildren were assessed by palpation, establishing a pre-intervention baseline for the prevalence of IDD.

This normal range has been extrapolated to the full population. It may be more logical to sample women of reproductive age, or adolescent girls thus providing more information on populations that may include those with or on the verge of greater need. The upper limit of the recommended range for these populations reflects concern about the risk of hyperthyroidism when high levels are introduced to a previously endemic population. Recent data have sµggested that the normal range for pregnant and lactating women should reflect their additional need and the risk that these needs may not be met if population levels are too low. However, this leaves a relatively narrow range for a median UI level that will both meet the needs for pregnant/lactating women, and not be excessive for the remainder of the population. This guide provides the best current estimates for the optimal values to meet the overall population needs.

Urinary iodine concentration is currently the most practical biochemical marker for iodine nutrition when carried out with appropriate technology and sampling. This approach assesses iodine nutrition only at the time of measurement, whereas thyroid size reflects iodine nutrition over months or years. Therefore, even though populations may have attained iodine sufficiency on the basis of median urinary iodine concentration, goitre may persist, even in children.

With rapid global progress in correcting iodine deficiency, examples of iodine excess are being recognized, particularly when salt iodization is excessive and poorly monitored. Tolerance to high doses of iodine is quite variable, and many individuals ingest amounts of several milligrams or more per day without apparent problems. The major epidemiological consequence

of iodine excess is iodine-induced hyperthyroidism (IIH) (Dunn, 2001). This occurs more commonly in older subjects with preexisting nodular goitres, and may occur even when iodine intake is within the normal range. Iodine intakes >300 μg/L per day should generally be discouraged, particularly in areas where iodine deficiency has previously existed. In these situations, more individuals may be vulnerable to adverse health consequences, including iodine-induced hyperthyroidism and autoimmune thyroid diseases.

In populations characterized by long-standing iodine deficiency and a rapid increase in iodine intake, median values for urinary iodine > 200 μg/L (and in pregnant women, above 250 μg/L) are not recommended because of the possible risk of iodine-induced hyperthyroidism. This adverse condition can occur during the 5-10 yrs following the introduction of iodized salt (Dunn, 2001). Beyond this period of time, median values up to 300 μg/L have not demonstrated side-effects, at least not in populations with adequately iodized salt. In schoolchildren, urinary iodine concentrations >500 μg/L are associated with increasing thyroid volume, which reflects the adverse effects of chronic iodine excess (Zimmermann et al., 2005).

Thyroid Size

Assessment of thyroid size by palpation is the time-honoured method of assessing IDD prevalence. However, because of the lack of sensitivity to acute changes in iodine intake, this method is of limited usefulness in assessing the impact of programmes once salt iodisation has commenced. In this case, urinary iodine is the most useful indicator because it is reflective of the current intake of iodine in the diet. The traditional method for determining thyroid size is inspection and palpation. Ultrasonography provides a more

precise and objective method. Both methods are described below. Issues common to palpation and ultrasound are not repeated in the section on ultrasound.

Thyroid Size by Palpation

The introduction of ultrasonography for the assessment of thyroid size has been a significant development. In areas of mild to moderate IDD, measurement of thyroid volume using ultrasound is preferable to palpation for grading goitre. New international reference values for thyroid volume by ultrasound have recently become available and can be used for goitre screening in the context of IDD monitoring (Dunn, 2001). The size of the thyroid gland changes inversely in response to alterations in iodine intake, with a lag interval that varies from a few months to several years, depending on many factors. These include the severity and duration of iodine deficiency, the type and effectiveness of iodine supplementation, age, sex, and possible additional goitrogenic factors. The term "goitre" refers to a thyroid gland that is enlarged. The statement that "a thyroid gland each of whose lobes have a volume greater than the terminal phalanges of the thumb of the person examined will be considered goitrous" is empiric, but has been used in most epidemiological studies of endemic goitre and is still recommended, Table 9.

Table 9: Simplified Classification of Goitrea by Palpation

Grade 0	No palpable or visible goiter
Grade 1	A goitre that is palpable but not visible when the neck is in the normal position (that is, the thyroid is not visibly enlarged) Thyroid nodules in a thyroid which is otherwise not enlarged fall into this category
Grade 2	A swelling in the neck that is clearly visible when the neck is in a normal position and is consistent with an enlarged thyroid when the neck is palpated

Source: WHO A Guide for Programme Managers 3rd ed. 2007

Palpation of the thyroid is particularly useful in assessing goitre prevalence before the introduction of any intervention to control IDD, but much less so in determining impact. Costs are associated with mounting a survey, which is relatively easy to conduct, and training of personnel. These costs will vary depending upon the availability of health care personnel, accessibility of the population, and sample size. Feasibility and performance vary according to target groups, as follows:

Neonates**:** It is neither feasible nor practical to assess goitre among neonates, whether by palpation or ultrasound. Performance is poor.

School-age children (6-12 yrs): This is the preferred group, as it is usually easily accessible. However, the highest prevalence of goiter occurs during puberty and childbearing age. Some studies have focused on children 8 to 10 yrs of age. There is a practical reason for not measuring very young age groups. The smaller the child, the smaller the thyroid, and the more difficult it is to perform palpation. If the proportion of children attending school is low, school children may not be representative. In these cases, spot surveys should be conducted among those who attend school and those who do not, to ascertain if there is any significant difference between the two. Alternatively, children can be surveyed in households.

Adults (Pregnant and lactating women) are of particular concern. Pregnant women are a prime target group for IDD control activities because they are especially sensitive to marginal iodine deficiency. Often they are relatively accessible given their participation in antenatal clinics. Women of childbearing age 15-44 yrs may be surveyed in households (WHO A Guide for Programme Managers, 2007).

The subject to be examined stands in front of the examiner, who looks carefully at the neck for any sign of visible thyroid enlargement. The subject is then asked to look up and thereby to fully extend the neck. This pushes the thyroid forward and makes any enlargement more obvious. Finally, the examiner palpates the thyroid by gently sliding their own thumb along the side of the trachea (wind-pipe) between the cricoid cartilage and the top of the sternum. Both sides of the trachea are checked. The size and consistency of the thyroid gland are carefully noted.

If necessary, the subject is asked to swallow (e.g. some water) when being examined the thyroid moves up on swallowing. The size of each lobe of the thyroid is compared to the size of the tip (terminal phalanx) of the thumb of the subject being examined. Goitre is graded according to the classification presented in Table 10 (WHO A Guide for Programme Managers, 2007).

A thyroid gland will be considered goitrous when each lateral lobe has a volume greater than the terminal phalanx of the thumbs of the subject being examined. The specificity and sensitivity of palpation are low in grades 0 and 1 due to a high inter-observer variation. As demonstrated by studies of experienced examiners, misclassification can be high. Another method is to stand behind the subject with the neck in the neutral position and hold the fingers (not thumb) over the area of the gland. The person is asked to swallow and the gland is palpated by the fingers as it glides up. This is repeated on each side of the neck. Table 10 gives the epidemiological criteria for establishing IDD severity, based on goitre prevalence in school-age children.

Table 10: Epidemiological Criteria for Assessing the Severity of IDD based on the Prevalence of Goitre in School-Age Children

	Degrees of IDD, expressed as percentage of the total of the number of children surveyed			
Total goitre rate (TGR)	None	Mild	Moderate	Severe
	0.0-4.9%	5.0-19.9%	20.0-29.9%	≥ 30%

Source: WHO A Guide for Programme Managers 3rd ed. 2007

The terms mild, moderate, and severe are relative and should be interpreted in context with information from other indicators. It is recommended that a total goitre rate or TGR (number with goiters of grades 1 and 2 divided by total examined) of 5% or more in schoolchildren 6-12 yrs of age be used to signal the presence of a public health problem. This recommendation is based on the observation that in normal, iodine-replete populations, the prevalence of goitre should be quite low. The cut-off point of 5% allows both for some margin of error of goitre assessment, and for goitre that may occur in iodine-replete populations due to other causes such as goitrogens and autoimmune thyroid diseases.

Goitre prevalence responds slowly to changes in iodine intake. Finally, in this context it is emphasized that thyroid size in the community may not return to normal for months or years after correction of iodine deficiency.

Thyroid Size by Ultrasonography

In areas of mild to moderate IDD, the sensitivity and specificity of palpation are poor and measurement of thyroid size using ultrasound is preferable. Ultrasonography is a safe, non-invasive, specialized technique that

can be quickly done (2-3 mins per subject) and is feasible even in remote areas using portable equipment. Ultrasonography provides a more precise measurement of thyroid volume compared with palpation. This becomes especially significant when the prevalence of visible goiters is small, and in monitoring iodine control programmes where thyroid volumes are expected to decrease over time. (WHO A Guide for Programme Managers, 2007)

Portable (weight 12-15 kg) ultrasound equipment with a 7.5 MHz transducer currently costs about US $15 000. A source of electricity is needed, and the operator needs to be specially trained in the technique. Differences in technique (e.g. the pressure applied with the transducer) and estimation of thyroid anatomy (e.g. inclusion of the thyroid isthmus and/or capsule thickness) can result in high inter-observer variability. Results of ultrasonography from a study population should be compared with reference data (Zimmermann et al., 2004). Reference values for thyroid volume measured by ultrasonography in schoolchildren of iodine-sufficient populations are shown in Table 11.

Table 11: Gender Specific 97th Percentile (P 97) of Thyroid Volume (mL) by Age and Body Surface Area (BSA) measured by Ultrasound in Iodine Sufficient 6–12 yr-old Children

	Boys	Girls		Boys	Girls
Age (yr)	P97	P97	BSA (m^2)	P97	P97
6	2.91	2.84	0.7	2.62	2.56
7	.29	3.26	0.8	2.95	2.91
8	3.29	3.76	0.9	3.32	3.32
9	4.19	4.32	1.0	3.73	3.79
10	4.73	4.98	1.1	4.20	4.32
11	5.34	5.73	1.2	4.73	4.92
12	6.03	6.59	1.3	5.32	5.61
			1.4	5.98	6.40
			1.5	6.73	7.29
			1.6	7.57	6.32

Source: Zimmermann, 2004

These are presented as a function of age, sex, and body surface area (BSA) in order to take into account the differences in body development among children of the same age in different countries. This approach is potentially useful in countries with a high prevalence of child growth retardation due to malnutrition with both stunting (low height-for-age) and underweight (low weight-for-age). An advantage of the thyroid volume-for-BSA is that the age of the child is not required, which in some populations is not known with certainty. A limitation of the thyroid volume-for-BSA is that it requires the collection of weights and heights: in severely malnourished populations of schoolchildren, 10% or more may have a BSA below the lowest BSA cut-off of 0.7. The thyroid volume references proposed here are applicable for goiter screening only if thyroid volume is determined by the standardized method described.

Blood Constituents

Other indicators include: thyroid stimulating hormone (TSH), and thyroglobulin (Tg). While TSH levels in neonates are particularly sensitive to iodine deficiency, and althoμgh difficulties in interpretation remain, there is a potential future for the use of neonatal TSH in the identification of IDD and their control; althoμgh the cost of implementing a TSH screening programme is too high for most developing countries. Measurement of Tg in children is a sensitive indicator of iodine status and improving thyroid function after iodine repletion. A standardized dried blood spot Tg assay has been developed and can be used for assessing and monitoring iodine nutrition in the field. Two blood constituents, TSH and Tg, can serve as surveillance indicators. In a

population survey, blood spots on filter paper or serum samples can be used to measure TSH and/or Tg.

Determining serum concentrations of the thyroid hormones, thyroxin (T4) and triiodothyronine (T3), is usually not recommended for monitoring iodine nutrition, because these tests are more cumbersome, more expensive, and less sensitive indicators. In iodine deficiency, the serum T4 is typically lower and the serum T3 higher than in normal populations. However, the overlap is large enough to make these tests impractical for ordinary epidemiological purposes.

1. Thyroid Stimulating Hormone (TSH)

The pituitary secretes TSH in response to circulating levels of T4. Serum TSH rises when serum T4 concentrations are low, and falls when they are high. Iodine deficiency lowers circulating T4 and raises the serum TSH, so iodine-deficient populations generally have higher serum TSH concentrations than do iodine-sufficient groups. However, the difference is not great and much overlap occurs between individual TSH values. Therefore, the blood TSH concentration in school-age children and adults is not a practical marker for iodine deficiency, and its routine use in school-based surveys is not recommended.

In contrast, TSH in neonates is a valuable indicator for iodine deficiency. The neonatal thyroid has a low iodine content compared to that of the adult, and hence iodine turnover is much higher. This high turnover, which is exaggerated in iodine deficiency, requires increased stimulation by TSH. Hence, TSH levels are increased in iodine-deficient populations for the first few weeks of life – this phenomenon is called transient hyperthyrotopinemia.

Impact Indicators

The indicators are shown in Table 12.

Table 12: Indicators of Impact at Population Level

Monitoring Indicator (units)	Age group for assessment	Advantages	Disadvantages
Median urinary iodine concentration (µg/L)	School-age children and pregnant women	– Spot urine specimens are easy to obtain – The most practical biochemical marker for iodine nutrition, when carried out with appropriate technology and sampling. – Feasible to process large numbers of samples at low cost – Cut-off points proposed for classifying iodine nutrition into different degrees of public health significance are well established – External quality control program in place	– Assesses iodine intake only over the past few days – Meticulous laboratory practice is required to avoid contamination with iodine – A sufficiently large number of samples must be collected to allow for varying degrees of subject hydration and other biological variations among individuals – Not valuable for individual assessment
Goitre rate by palpation (%)	School-age children	– Simple and rapid screening test – Requires no specialized equipment assessed	– Specificity and sensitivity of palpation are low in grades 0 and 1 due to a high inter-observer variation – Responds slowly to changes in iodine intake

Table 12: continued

Goitre rate assessed by ultrasound (%)		– A more precise measurement of thyroid volume compared with palpation – Safe, non-invasive	– Expensive equipment and a source of electricity is needed – operator needs to be specially trained in the technique
	School-age children	– International reference values for thyroid volume in schoolchildren are available as a function of age, sex, and body surface area	– Responds slowly to changes in iodine intake
TSH (mIU/L)	Newborns	– Measures thyroid function at a vulnerable age when iodine deficiency directly affects the developing brain – If screening programs to detect congenital hypothyroidism is in place then only additional cost will be for data analysis – Collection by heel stick and storage on filter paper is simple – Blood spots can be stored for several weeks at cool, dry room temperatures	– Not recommended to be set up solely to assess community iodine deficiency due to expense – Cannot be used when antiseptics containing iodine are used during delivery – Requires use of a standardized, sensitive assay – Should be taken either from the cord at delivery or by heel prick at least 48 hours after birth to avoid physiological newborn surge
Tg (µg/L)	School-age children	– Collection by finger stick and storage on filter paper is simple – Can be stored for several weeks at cool, dry room temperatures, so sampling practical even in remote areas – Measures improving thyroid function within several months after iodine repletion	– Expensive immunoassay – Requires laboratory infrastructure

Table 12: continued

	– Standard reference material is now available, but needs to be validated – An international reference range has been established

Source: WHO A Guide for Programme Managers, 2007

To assess iodine status, and to monitor and evaluate the impact of salt iodisation on the population, median urinary iodine is the main indicator to be used. Goitre assessment by palpation or by ultrasound may be useful in assessing thyroid function, but is difficult to interpret once salt iodization has started. The measurement of thyroid stimulating hormone (TSH) levels in neonates where a screening programme is in place, and of thyroglobulin in school-age children where feasible are both useful indicators of thyroid function. Once a salt iodisation programme has been initiated, the principal impact indicator recommended is the population median urinary iodine level. Changes in goitre prevalence lag behind changes in iodine status, and therefore cannot be relied upon to accurately reflect current iodine intake.

Control of Iodine Deficiency Disorders

The recommended strategy for IDD control is based on correcting the deficiency by increasing iodine intake through supplementation or food fortification.

Correction of Iodine Deficiency

Universal Salt Iodisation (USI)

In 1994, a special session of the WHO and UNICEF Joint Committee on Health Policy recommended USI as a safe, cost-effective, and sustainable strategy to ensure sufficient intake of iodine by all individuals..In nearly all

countries where iodine deficiency occurs, it is now well recognized that the most effective way to achieve the virtual elimination of IDD is through USI. Universal salt iodisation is defined as when all salt for human and animal consumption is iodised to the internationally agreed recommended levels elimination. In those countries, the emphasis will shift to ensuring that these achievements are permanently sustained. USI involves the iodisation of all human and livestock salt, including salt used in the food industry. Adequate iodization of all salt will deliver iodine in the required quantities to the population on a continuous and self-sustaining basis (Iodine Status Worldwide, 2004).

USI, which ensures that all salt for human and animal consumption is adequately iodised, has been remarkably successful in many countries. Over 30 countries have achieved the goal of USI (>90% of households using iodised salt), and many others are on track. Most countries that have failed to achieve coverage over 20% have conflict situations that hinder all health efforts. In rare instances, it may happen that salt iodisation efforts are unable to meet the requirement of women during pregnancy, exposing the progeny to potential developmental risks. In such situations, while efforts to improve the salt iodization programme continue, iodine supplementation may be considered for both pregnant women and children less than two years of age as a daily oral dose of iodine or a single oral dose of iodised oil every 6-12 mths (WHO/UNICEF/ICCIDD, 2004).

There is much evidence that correction of iodine deficiency has been followed by a revival of a community suffering from the effects on the brain of hypothyroidism due to iodine deficiency. Such an increase in vitality is

responsible for improved learning by schoolchildren, improved work performance of adults, and a better quality of life. The economic significance of the prevention of iodine deficiency disorders needs to be clearly understood (Zimmermann, 2008). Education about these basic facts has to be repeated, with the inevitable changes over time in Ministries of Health and the public health community and salt producers. Otherwise, a successful programme will lapse, as has occurred in a number of countries.

Iodine Supplementation

The first iodine supplements were in the form of an oral solution of iodine such as Lugol, which was given daily. After the Second World War, considerable progress was made in reducing IDD with iodised oil initially using the intramuscular form and in the 1990s, using the oral form. For example, iodised oil was used with success in Papua New Guinea and thereafter in China, several countries in Africa and Latin America and in other severely endemic areas.

The oral form of iodised oil has several advantages over the intramuscular form: it does not require special storage conditions or trained health personnel for the injection and it can be given once a year. Compared to iodised salt, however, it is more expensive and coverage can be limited since it requires direct contact with each person. With the introduction of iodised salt on a large scale, iodised oil is now only recommended for populations living in severely endemic areas with no access to iodised salt.

In some countries and areas with insufficient access to iodised salt, additional temporary strategies need to be considered to ensure optimal iodine nutrition for these groups while strengthening the salt iodisation programmes

to reach universal coverage (WHO/UNICEF/ICCIDD, 2007). In particular, each country should assess the current situation of its salt iodisation programme to identify national or subnational problems and to update its strategies and action plans. The most vulnerable groups, pregnant and lactating women, should be considered for supplementation with iodine, Table 13.

Table 13: Recommended Dosages of Daily and Annual Iodine Supplementation

Population group	Daily dose of iodine supplement (μg/dy)	Single annual dose of iodised oil supplement (μg/yr)
Pregnant women	250	400
Lactating women	250	400
Women of reproductive age (15-49 yrs)	150	400
Children < 2 yrs[a.b]	90	200

Source: WHO A Guide for Programme Managers 3rd ed. 2007

Until the salt iodisation programme is scaled up. For children 7-24 mths of age, either supplementation or use of iodine-fortified complementary foods may be a possible temporary public health measure. For children 0-6 mths of age, iodine supplementation should be given through breast milk. This implies that the child is exclusively breastfed and that the lactating mother received iodine supplementation as indicated above. These figures for iodine supplements are given in situations where complementary food fortified with

iodine is not available, in which case iodine supplementation is required for children of 7-24 mths of age (WHO/UNICEF/ICCIDD, 2007).

Over the past century, many food vehicles have been fortified with iodine: bread, milk, water and salt. Salt is the most commonly used vehicle. It was first introduced in the 1920s in the United States and in Switzerland. However, this strategy was not widely replicated until the 1990s when the World Health Assembly adopted Universal Salt Iodisation (USI) as the method of choice to eliminate IDD (Bath, 2015).

In 2002, at the Special Session on Children of the United Nations (UN) General Assembly, the goal to eliminate IDD by the year 2005 was set. USI was chosen as the best strategy because salt is one of the few commodities consumed by everyone, consumption is fairly stable throughout the year, and production is usually in the hands of few producers. Moreover, the iodisation technology is easy to implement and available at a reasonable cost. Again, the addition of iodine to salt does not affect its colour, taste or odour; the quality of iodised salt can be monitored at the production, retail and household levels. The price of salt is inexpensive worldwide (WHO/UNICEF/ICCIDD, 2007).

Table salt is a critical component in the human diet, required for the correct functioning of nerves and muscles, the absorption of nutrients from the small intestines to maintain the correct balance of fluids within the body. Iodine as potassium iodate (KIO_3) or potassium iodide (KI) is a key component that may be present in table salt to fight IDDs. Ordinary table salt contains 40% sodium, the brain continually monitors the amount of salt present in our bodies and sends instructions to the kidneys to either reabsorb or remove sodium as appropriate. The body of a typical male weighing 70 kg

contains about 92 g of sodium. Salt depletion is usually associated with dehydration an active worker can lose up to 8 L of sweat/dy, and a failure to replenish the salt lost can have harmful effects on the body (WHO/UNICEF/ICCIDD, 2007).

In order to meet the iodine requirements of a population it is recommended to add 20-40 ppm of iodine to salt (assuming an average salt intake of 10 g per capita/dy) (WHO/UNICEF/ICCIDD, 2007). There are two forms of iodine fortificants, potassium iodate and potassium iodide. Because iodate is more stable under extreme climatic conditions it is preferred to iodide, especially in hot and humid climates (Bath, 2015).

Evaluation of the Correction Measures

Monitoring Iodine Levels of Salt

An IDD control programme based on salt iodisation clearly cannot succeed unless all salt for human consumption is being adequately iodised. Therefore, the most important indicator to monitor is salt, and the most important place to monitor salt is at the site of production and importation. If all salt leaving production facilities and imported salt is properly iodised, packaged, and labelled, populations consuming this salt are likely to have their iodine requirements met (WHO/UNICEF/ICCIDD, 2007).

There is no prescribed sampling method at this level, and countries have widely variable capacity and have used many approaches. Ideally, for any given district, there is enough market samples tested on an annual basis to determine the degree to which non-iodised salt remains available in the marketplace, thus giving some perspective to household coverage figures (WHO/UNICEF/ ICCIDD, 2007). Salt from each selected household should

be tested. Testing using routine test kits will provide an estimate of the percent of households using salt with no iodine, but will not provide accurate information on the percent using adequately iodised salt, or information on salt with excessive iodine. Thus, titration or another quantitative method should be performed on at least a sub-sample of households for any coverage survey. (WHO/ UNICEF/ICCIDD, 2007).

Governments usually set the level at which salt should be iodized. Monitoring aims to ensure that the salt industry complies with the regulations set by the government and that the iodine levels are re-adjusted if necessary. Iodine levels are monitored (at a minimum) at the factory and household levels, and if possible at the retail level. If iodised salt is imported it is monitored at the point of entry into the country. The monitoring process at the factory level is the salt producer's or importer's responsibility and is regularly supervised by the relevant public authorities (WHO A Guide for Programme Managers, 2004).

In most cases the Ministry of Health carries out the monitoring at the household level. Iodine content in salt is best measured by titration. Field test kits have been developed to only give qualitative results, indicating if iodine is present or not. Because of this, they are of limited use and their reliability has recently been questioned. However, they can still be useful for training, education and for advocacy purposes for the public and staff.

Monitoring of iodine status of populations (WHO A Guide for Programme Managers, 2004).

Assessing iodine status provides information on whether there is adequate iodine intake in the population surveyed. Iodine status is the most

immediate measure of whether the thyroid gland has adequate iodine to function normally and protect the individual from the manifestations of iodine deficiency. The median urinary iodine concentration reflects population status and is the indicator most commonly assessed. Iodine status assessment may be less frequent since it involves the collection of urine, and therefore requires more financial and human resources. Follow-up urinary iodine assessments are generally performed after intervention programs achieve a certain level of success (WHO/UNICEF/ICCIDD, 2007).

Urinary iodine is frequently assessed through school surveys (since this is an efficient way to estimate the household iodine nutrition situation) or through overall population assessments. While the median value in a representative sample of schoolchildren or the general population provides a reasonable population estimate, it may not reflect the situation in pregnant women, whose iodine requirements are greater. Sampling of pregnant women can be difficult because the number of pregnant women present in household-based surveys may be small. Assessing the median value in women of reproductive age or among adolescent girls is more feasible in a population-based survey, and may be helpful in interpreting the median population value (WHO A Guide for Programme Managers, 2004).

Ideally, assessment of iodine status should include concurrent assessment of household use of iodised salt. This provides information on both the likely iodine intake and iodine status, making it easier to distinguish between difficulties with iodised salt quality, and iodised salt use. When adequately iodised salt is used, this should be reflected in adequate iodine

status in the population sampled (WHO A Guide for Programme Managers, 2004).

Thyroid Function Assessment

Assessing thyroid function provides information on whether the thyroid gland is responding to adequate iodine intake, and is the ultimate measure of whether a population is protected from iodine deficiency. Thyroid function reflects the ability of the thyroid to produce thyroid hormone, which is essential for normal development.

Monitoring and Evaluating the IDD Control Programmes

As programmes mature, it is important to understand their vulnerability and whether they are likely to be sustained. A number of criteria have been established to determine whether elimination goals have been met, and a series of programmatic indicators have been developed to help understand the likelihood that a program will be sustained. In considering whether the sustainable elimination of iodine deficiency as a public health problem has been achieved, the following criteria should be met.

With regard to salt iodisation

Availability and use of adequately iodised salt (>20 and <40 ppm) must be guaranteed. This is demonstrated by its use by more than 90% of households, Table 18. Conditions demonstrating successful use of salt as vehicle for eliminating IDD are 95% of salt for human consumption must be iodised according to government standards for iodine content as determined by titration, at the production or importation levels. The percentage of food-grade salt with iodine content of between 20 and 40 ppm in a representative sample

of households must be equal to or greater than 90% as determined by rapid test kits (RTK) and by titration in a sub-sample (WHO/UNICEF/ICCIDD, 2007):

With regard to the population's iodine status

The median urinary iodine concentration in the general population should be within the range 100-199 μg/L, Table 14. The median urinary iodine concentration in the pregnant women population should be within the range 150-249 μg/L, Table 14. The most recent monitoring data (national or regional) should have been collected within the last five years (WHO/UNICEF/ICCIDD, 2007).

Table 14: Summary of Criteria for Monitoring Progress towards Sustainable Elimination of IDD as a Public Health problem

Indicator	Goals
Salt iodisation:	
Proportion of households using adequately iodized salt	>90%
Urinary iodine:	
Median in the general population	100-199 μg/ L
Median in pregnant women	150-249 μg/L
Programmatic indicators 1. National body responsible to the government of IDD elmination. It should be multidisciplinary, involving the relevant fields of nutrition, medicine, education, the salt industry, the media, and consumers, with a chairman appointed by the Minister of Health. 2. Evidence of political commitment to USI and elmination of IDD; 3. Appointment of a responsible executive officer for the IDD elmination programme; 4. Legislation or regulation of USI; 5. Commitment to regular progress in IDD elmination, with access to laboratories able to provide accurate data on salt and urinary iodine;	Attainment of 8 out of 10 indicators

Table 4: Continued

6. A programme of public education and social mobilisation on the importance of IDD and the consumption of iodised salt; 7. Regular data on iodised salt at the factory, retail and household levels; 8. Regular laboratory data on urinary iodine in school-age children, with appropriate sampling for higher-risk areas; Co-operation from the salt industry in maintenance of quality control and; database for recording results or regular monitoring procedures particularly for salt iodine, urinary iodine and if available, neonatal thyroid stimulating hormone (TSH), with mandatory public reporting.	

Source: WHO A Guide for Programme Managers 3rd ed. 2007

With regard to the programmes

Presence of a national multi-sector coalition responsible to the government for the national programme for the elimination of IDD with the following characteristics; national stature, (all concerned sectors, including the salt industry, represented with defined roles and responsibilities convenes at least twice yearly to demonstrate political commitment), enact legislation, establish methods for assessment of progress in the elimination of IDD (WHO/UNICEF/ICCIDD, 2007).

There is a need for periodic review of the entire programme, with the help of WHO, UNICEF, ICCIDD, and other appropriate organisations involved in IDD elimination. Such external evaluation provides independent assessment, which is extremely helpful to a country programme. It can also provide programmes with reassurance of their performance and effectiveness. For acknowledgement of attainment of the sustainable elimination of IDD, countries may request an evaluation through UNICEF, WHO, or ICCIDD country offices. As a result, the mental ability of ostensibly normal children

and adults living in areas of iodine deficiency is reduced compared to what it would be otherwise (WHO/UNICEF/ICCIDD, 2007).

Thus, the potential of a whole community is reduced by iodine deficiency. Where the deficiency is severe, there is little chance of achievement and underdevelopment is perpetuated. Indeed, in an iodine-deficient population, everybody may seem to be slow and rather sleepy. The quality of life is poor, ambition is blunted, and the community becomes trapped in a self-perpetuating cycle. Even the domestic animals, such as village dogs, are affected. Livestock productivity is also dramatically reduced (Zimmermann, 2008; WHO/UNICEF /ICCIDD, 2007).

Sustaining IDDs Control Programmes

This involves a combination of median urinary iodine levels in the population, availability of adequately iodised salt at the household level, and a set of programmatic indicators which are regarded as evidence of sustainability. The progress made with IDD programs in the past decade reflects program maturation, and raises the question of how well these programs will be sustained into the future. IDD cannot be eradicated in one great global effort like smallpox and poliomyelitis, since these are infectious diseases with only one host: man. Once eliminated, they cannot come back. By contrast, IDD is a nutritional deficiency that is primarily the result of deficiency of iodine in soil and water (WHO/UNICEF/ICCIDD, 2007).

IDD can therefore return at any time after their elimination if program success is not sustained. Indeed, there is evidence that iodine deficiency is returning to some countries where it had been eliminated in the past (WHO/UNICEF/ICCIDD, 2007). Ideally, salt iodisation programmes ensure

that there is adequate iodine intake for the entire population, and the cost of iodisation is included as part of the cost of doing business within the salt industry. The IDD program in this case simply needs to monitor the situation. In reality, even with mature salt iodisation programmes with high coverage, programs remain vulnerable to changes in the salt industry, changes in political will, and changes in awareness or consumer acceptance. Thus, it is important to monitor the overall programmatic indicators as well as measures of salt iodization and impact to ensure that achievements are sustained (WHO/UNICEF/ICCIDD, 2007).

In order to achieve the global goal set for 2005, IDD control programmes and monitoring need to be constantly sustained due to the fact that IDD simply re-appears if salt iodisation is interrupted. This may happen when the responsible public health authorities are demobilised or if the salt industry fails to effectively monitor iodine content. In order to assess the sustainability of control programmes and track their progress towards the IDD elimination goal, criteria have been established by WHO, Table 15.

Table 15: Criteria for Monitoring Progress towards Sustainable Iodine Deficiency Disorders Elimination

Indicators	Goals
1. Salt iodisation coverage	>90%
2. Proportion of households consuming adequately iodised salt[a]	
3. Urinary iodine	
4. Proportion of population with urinary iodine levels below 100 μg/L	<50%
	<20%
5. Proportion of population with urinary iodine levels below 50 μg/L	

Table 15: Continued

6. Programmatic indicators 7. National body responsible to the government of IDD elmination. It should be multidisciplinary, involving the relevant fields of nutrition, medicine, education, the salt industry, the media, and consumers, with a chairman appointed by the Minister of Health. 8. Evidence of political commitment to USI and elmination of IDD; 9. Appointment of a responsible executive officer for the IDD elmination programme; 10. Legislation or regulation of USI; 11. Commitment to regular progress in IDD elmination, with access to laboratories able to provide accurate data on salt and urinary iodine; 12. A programme of public education and social mobilisation on the importance of IDD and the consumption of iodised salt; 13. Regular data on iodised salt at the factory, retail and household levels; 14. Regular laboratory data on urinary iodine in school-age children, with appropriate sampling for higher-risk areas; 15. Co-operation from the salt industry in maintenance of quality control and; 16. A database for recording results or regular monitoring procedures particularly for salt iodine, urinary iodine and if available, neonatal thyroid stimulating hormone (TSH), with mandatory public reporting.	At least 8 of the 10

Source: WHO/UNICEF/ICCIDD, 2007

Adequately iodised salt refers to at least 15 ppm at household level

Effort of International Bodies (Global Occurrence of IDDs)

The WHO has estimated that there are throughout the world 200 million persons with goitre in 1968. The ICNND, which has studied nutritional problems of military and civilian populations in 25 countries, found goiter to be a general nutritional condition in the West Indies, and Colombia WHO/UNICEF/ICCIDD, 2007. The World Summit for Children in 1990, and consequently the International Conference on Nutrition in 1992 postulated the elimination of IDD by the year 2000, whilst the year 1995 was set for achievement of USI in Ghana under the supervision of ICCIDD (Ghana Nutritions Profile, 2011). Between 1994 and 2006, the number of countries that carried out a urinary iodine national survey increased to 94, and survey data on iodine deficiency now covers 91.1% of the world population (WHO/UNICEF/ICCIDD, 2007).

There is still no data for 63 countries, which together represent 8.9% of the world population. Out of the 130 countries, there are only 47 countries where IDD still remains as a public health problem, compared to 54 in 2004 and 126 in 1993. Iodine intake (reflected by the median urinary iodine concentration) in the other 83 countries is as follows: “adequate” or “above recommended nutrient intakes” in 76 countries; and “excessive” in seven countries. About 31% (900.9 million) of the world population is estimated to have insufficient iodine intakes, with the most affected WHO regions being South-East Asia and Europe as shown in Table 10. It is currently estimated that 70% of households throughout the world have access to (and use) iodised salt (WHO/UNICEF /ICCIDD, 2007).

The Prevalence of IDD

In Ghana, loss of productivity to prevalence of IDD is about $22 m annually. The prevalence if continues, the population could be incapacitated in taking decision thereby blocking human and social development (Ghana Nutritions Profile, 2011). The following are some of the reasons for increasing IDDs globally according to Tenpenny, (2014):

1. Decrease in salt consumption for fear of hypertension,
2. Decrease in egg consumption for fear of cholesterol,
3. Decrease in fish consumption for fear over Hg,
4. Minimal access to sea vegetable like sea weed,
5. Soil depletion and minerals depletion caused by accelerated deforestation and erosion
6. Variety in the amount of the I_2 added during the iodisation process,
7. Loss of I_2 due to salt impurities and lack of chemical body
8. Varying dimension of I_2 in salt batches and in individual packages,
9. Loss of I_2 during cooking ranges from 50-70%,
10. Loss of I_2 during storage (exposure to sun, etc.) or at the kitchen counter ranges from 10-100%.
11. There is also the effect of goitrogenic foods

The Benefits of Iodised Salt in Prevention of Iodine Deficiency Disorders

In order to combat IDD, iodised table salt has been used in many countries since the 1920s. It can contribute significant amounts of iodine to daily intakes, although it is not universally available, nor its fortification mandatory in many countries. Iodine in iodised salt is 100% bioavailable

(Stephanie, 2014). In practice, the iodisation of salt has proven to be the best method of iodine supplementation (L'Abbe, 2003).

Stabilizing Iodised Salt

Dextrose is added to the salt as a stabilizer, whilst calcium silicate is added to prevent clumping. The iodised salt may volatilise and thus sodium carbonate is sometimes added. The iodate has been found to be better adopted for fortification than the iodide since it is a more stable compound, especially in areas where moisture and high temperatures prevail (Stephanie, 2014).

Factors that Determine Salt Iodine Content

The regulatory framework represents the primary factor that determines the iodine content of salt in any country. Salt is iodised by the addition of fixed amounts of potassium iodate (KIO_3) or potassium iodide (KI), as either a dry solid in a powder form or an aqueous solution, at the point of production. The amount added to salt should be in accordance with the regulation of the specific country where it will be used. Iodate is recommended as fortificant in preference to iodide because it is much more stable (WHO/UNICEF/ICCIDD, 2007).

The stability of iodine in salt and levels of iodisation are issues of crucial importance to national health authorities and salt producers. They have implications for programme effectiveness, safety, and cost. The actual availability of iodine from iodised salt at the consumer level can vary as a result of variability in the amount of iodine added during the iodisation process; uneven distribution of iodine in the iodised salt, and/or variation in particle size of salt crystals in a batch or bag (Stephanie, 2014).

The stability also depends on the extent of loss of iodine due to salt impurities, packaging and environmental conditions during storage and distribution; loss of iodine due to processing; cooking processes in the household; and the availability of non-iodised salt from unconventional marketing sources. In order to determine appropriate levels of iodisation, an accurate estimate within countries is required of the losses of iodine occurring under local conditions between the time of iodisation and the time of consumption. Control of moisture content in iodised salt throughout manufacturing and distribution is critical to the stability of the added iodine. Considerable losses of iodine (30-80%) resulting from high humidity and porous packaging materials can be significantly reduced with packaging which provides a good moisture barrier. This would prevent iodine losses regardless of climatic conditions (WHO/UNICEF/ICCIDD, 2007; Joost, 2006).

Factors affecting the utilisation of iodised salt, should be reassessed focusing on the percentage of households using adequately iodised salt; production-level quality assurance; factors affecting the iodine content of salt such as packaging, transport, and storage; and food habits in relation to salt intake and cooking practices.

National authorities should establish initial levels for iodisation, and regular surveys of salt iodine content and urinary iodine levels to determine if the programme is having the desired effect. Discussions and regulations about iodine levels in salt must clearly specify whether they refer to total content of iodine alone or to content of iodine compound (KIO_3 or KI). It is recommended that the level be expressed as content of iodine alone. This

approach emphasizes the physiologically important component (iodine) and facilitates comparison of its different forms.

Managing the Iodised Salt Program in a Country

For optimal management and functioning of the salt iodisation programme in a country, all the partners involved in IDD control should take responsibility for coordinating and driving IDD-related activities in the country (WHO/UNICEF/ICCIDD, 2007).

Heavy Metals Contamination of Iodised Salt

Heavy metal contamination of iodised salt could be a health hazard. Some of the most toxic metals found in the ocean and are detrimental to the health of consumers of edible salts are cadmium, iron, lead, zinc, cobalt and aluminium (Kulkani et al., 2013; Kwong et al., 2004).

Cadmium is an extremely toxic metal. Overexposure may cause fatigue, headaches, nausea, vomiting, abdominal cramps, diarrhea, and fever. In addition, progressive loss of lung function (emphysema), abnormal buildup of fluid within the lungs (pulmonary edema), and breathlessness (dyspnea) may also be present. In some cases, affected individuals may exhibit increased salivation; yellowing of the teeth; an unusually rapid heart beat (tachycardia); low levels of iron within the red blood cells (anemia); bluish discoloration (cyanosis) of the skin and mucous membranes due to insufficient oxygen supply to these tissues; and/or an impaired sense of smell (anosmia) (Kulkani et al., 2013; Kwong et al., 2004).

Individuals with cadmium poisoning may also experience improper functioning of the canals with the kidney (renal tubular dysfunction) characterized by excretion of abnormally high levels of protein in the urine

(proteinuria), minor changes in liver function, and/or softening of certain bones (osteomalacia) (Kulkani et al, 2004). The presence of cadmium of in iodised salt may originate from the source from which the salt was derived, for instance, sea salt could be contaminated if the sea is polluted.

Iron poisoning is an iron overload caused by a large excess of iron intake. Lead poisoning is a medical condition in humans caused by increased levels of lead in the body. Lead interferes with a variety of body processes and is toxic to many organs and tissues including the heart, bones, intestines, kidneys, and reproductive and nervous systems. It interferes with the development of the nervous system and is therefore particularly toxic to children, causing potentially permanent learning and behavior disorders. Symptoms include abdominal pain, confusion, headache, anemia, irritability, and in severe cases seizures, coma, and death (Kulkani et al., 2013; Kwong et al., 2004).

In children, symptoms vary depending upon the degree of exposure to lead. Some affected individuals may not have any noticeable symptoms. Symptoms usually develop over a three to six week time period. Lead overexposure may cause children to be less playful, clumsier, irritable, and sluggish (lethargic). In some cases, symptoms include headaches, vomiting, abdominal pain, lack of appetite (anorexia), constipation, slurred speech (dysarthria), changes in kidney function, unusually high amounts of protein in the blood (hyperproteinemia), and unusually pale skin (pallor) resulting from a low level of iron in the red blood cells (anemia). Neurological symptoms associated with lead overexposure include an impaired ability to coordinate voluntary movements (ataxia), brain damage (encephalopathy), seizures,

convulsions, swelling of the optic nerve (papilledema), and/or impaired consciousness. Some affected children experience learning or behavioral problems such as mental retardation and selective deficits in language, cognitive function, balance, behavior, and school performance. In some cases, symptoms may be life-threatening (Kulkani et al., 2013; Kwong et al., 2004).

In adults, overexposure to lead may cause high blood pressure and damage to the reproductive organs. Additional symptoms may include fever, headaches, fatigue, sluggishness (letheragy), vomiting, loss of appetite (anorexia), abdominal pain, constipation, joint pain, loss of recently acquired skills, incoordination, listlessness, difficulty sleeping (insomnia), irritability, altered consciousness, hallucinations, and/or seizures. In addition, affected individuals may experience low levels of iron in the red blood cells (anemia), peripheral neuropathy, and, in some cases, brain damage (encephalopathy) (Kulkani et al., 2013; Kwong et al., 2004).

Some affected individuals experience decreased muscle strength and endurance; kidney disease; wrist drop; and behavioral changes such as hostility, depression, and/or anxiety. In some cases, symptoms may be life-threatening (Kulkani et al., 2013; Kwong et al., 2004). Lead in iodised salt could also originate from polluted sea from which it was produced.

Even though zinc is an essential requirement for a healthy body, excess zinc can be harmful, and cause zinc toxicity. Such toxicity levels have been seen to occur at ingestion of greater than 225 mg of Zinc. Excessive absorption of zinc can suppress copper and iron absorption. The free zinc ion in solution is highly toxic to bacteria, plants, invertebrates, and even vertebrate fish (American College of Medical Toxicology, 2013).

Cobalt, used in making jet engines, may cause nausea, vomiting, lack of appetite (anorexia), ear ringing (tinnitus), nerve damage, respiratory diseases, an unusually large thyroid gland (goiter), and/or heart and/or kidney damage. (Eicher & Avery , 2005)

Aluminium has no known biological role and its classification into toxic metals is controversial. It is not a heavy metal, but it is included in this discussion because producers of iodated salt use aluminium pans in carrying the salt during production. Common symptoms of poisoning may include gastrointestinal, renal, and neurological symptoms, such as headaches, irritability, psychosis, stupor, coma, and convulsions. Aluminum containers used in the manufacture and processing of some foods, cosmetics and medicines, and also for water purification may cause poisoning. Overexposure to aluminum may cause brain damage (encephalopathy) (Kulkani et al., 2005).

Several studies have been conducted on the levels of heavy metals in iodised salt. In one of the studies to determine the levels of heavy metals in table and bakery refined salts, 81 table refined salt samples and the same number of bakery refined salt samples were purchased from retail market in the province of Hamadan, Iran. The levels of lead (Pb), cadmium (Cd), mercury (Hg), copper (Cu), and iron (Fe) were then determined using atomic absorption spectroscopy method. The levels (mean ± SD µg/g) of Pb, Cd, Hg, Cu, Fe in table refined salt samples were 0.852 ± 0.277, 0.229 ± 0.012, 0.054 ± 0.040, 1.25 ± 0.245 and 0.689 ± 1.58, respectively. The results for the same metals in bakery refined salt samples were as follows (mean ± SD µg/g): 22 ± 0.320 for Pb, 0.240± 0.018 for Cd, 0.058 ± 0.007 for Hg, 1.89 ± 0.218 for Cu, and 8.75 ± 2.10 for Fe. Heavy metal concentrations were generally higher in

bakery refined salt. All values for these metals in the table and bakery refined salts were lower than the permitted consumption level defined by Codex (2 µg/g of Pb, 0.5 µg/g of Cd, 0.1 µg/g of Hg, and 2 µg/g of Cu). (Hashmati & Iraj, 2018; Kulkani et al., 2013).

Analytical Methods for the Determination of Iodine in Iodised Salt

There are various analytical methods for determining iodine in iodised salt samples. Some of the recent methods include Kinetic Spectrophotometric Methods (Ni & Wang, 2007). Flow Injection Analysis (Shabarn et al., 2011), Microspectrophotometry after Liquid Phase Microextraction (Pereira et al; 2010), Using Cds Quantum Dots as Fluorescence Probes (Tang, et al., 2010), liquid–liquid extraction Microexratcation by High-Performance Liquid Chromatography-Diode, array Detection (Gupta et al., 2011), Ion Chromatography with Integrated Amperometric Detection (Babulai, et al., 2010), Transient Isochophoresis Capillary Zone (Tang et al., 2009), Gas Chromatography-Mass Spectrophotometry (Das et al., 2004), Using Polymer Membrane Selective for molecular iodine (Bhaget et al., 2008), A Neutron Activation Analysis Method (Bhaget et al., 2009), A Non Suppressed Ion Chromatography with Inductively Coupled Mass Spectroscopy (ICP-MS) (Preestis et al., 2003).

Several other analytical techniques have been applied to determine iodine in iodised salt. These include Inductively Couple Plasma Mass Specttrophometry (ICPMS), Radiochemical Neutron Activation Analysis and Capillary Electrophoresis (Kharzan et al., 2013).

The iodine content of salt has also been determined quantitatively with the classical titration method, and qualitatively using rapid test kits. In

addition to the titration method, technology has advanced the possibilities of analyzing the iodine content of salt quantitatively using potentiometry or spectrophotometry. All of these methods have certain advantages and disadvantages which generally influence the choice of method in specific circumstances. However, the titration method, which is by far the most commonly used quantitative method, still remains the reference method by WHO for determining the iodine concentration in salt. (WHO A Guide for Programme Managers, 2007).

Classical Titration Method

It is most frequently used because of accuracy, relative ease of use and low cost of operation. Depending on the form of iodine (iodate or iodide) added to the salt, different salt iodine testing methods are needed. If a salt sample is fortified with the KI, the iodometric method will not detect iodine content and vice versa. In cases of unknown form of the iodine in salt, a simple spot check method can be used for verification (Delange, 2001). The titration method requires the use of a small laboratory equipped with some basic instruments, such as a precision scale, a burette, glassware, and pipettes. Additional equipment, such as a magnetic stirrer and dispensers, will save time and optimise the analytical procedure.

Basically, iodine analysis by titration involves the preparation of solutions and a standard solution which will last for variable periods of time, and then determining the iodine concentration in a salt solution by adding the pre-made reagents/solutions followed by the titration step.

Specifically, the iodine content of salt is determined by liberating iodine from salt and titrating the liberated iodine with sodium thiosulfate

solution using starch as an external indicator. The method of liberating iodine from salt varies depending on whether salt is iodised with iodate or iodide. The procedure requires some training and laboratory skills, which can be conveyed to salt producers during a training course. Titration, or an equivalent method, is preferred for accurate testing of salt batches produced in factories or upon their arrival in a country, and in cases of doubt, contestation, etc. This method is recommended for determining the concentration of iodine in salt at various levels of the distribution system where such accurate testing is required, and for testing when there are legal enforcement issues. Once the method is established, it is necessary to adhere to proper internal and external quality control measures (WHO A Guide for Programme Managers, 2007).

Rapid Test Kits (RTK)

These are small 10-50 mL bottles containing a stabilized starch-based solution. One drop of the solution dripped on a teaspoon of salt containing iodine produces a blue/purple colour change. Colouration indicates that iodine is present. Different test kits are used depending on whether the salt is iodized with potassium iodate or iodide. In cases where there is suspicion of alkalinity in the salt sample, a 'recheck solution' is used.

A drop of this solution is applied first, followed by the test solution. Recent evaluations of these kits showed that the colour reaction cannot be used as a quantitative indication of the iodine conte (WHO A Guide for Programme Managers, 2004). These kits should therefore be regarded as qualitative rather than quantitative and are most appropriate to indicate the presence or absence of iodine, but not of the concentration. An advantage of rapid test kits is that they can be used in the field to give an immediate result.

They are therefore useful to health inspectors and others who are involved in carrying out spot checks on food quality or household surveys. They may also play a valuable educational role, in that they provide a visible indication that salt actually is iodised. Accordingly, they can be used for demonstration purposes in schools and other institutions. However, because rapid test kits do not give a reliable estimate of iodine content (Zimmermann, 2008), results must be backed up by titration.

Determination of Microbes in Salt

Total Viable Count (TVC)

TVC is the quantitative estimates of the concentration of microorganisms (such as bacteria, yeast and mold and spores) in a sample. The count represent the number of colony forming units (CFU) per g (or per mL) of the sample.

Total Coliform

Include bacteria that are found in the soil, in water that has been influenced by surface water, and in human or animal waste.

Analytical Methods for the Determination of Iodine in Urine

Several methods have been developed to determine urinary iodine, this is important in assessing the iodine nutritional status of a population and subsequently used to define, indicate, survey and monitor iodine deficiency, and its treatments (Soldin, 2002).

Urine is the preferred sample for in vitro analyses of iodine, although other sample types, such as feces, tissue, blood, serum, and hair, can also be used on a more limited basis with good detection sensitivities that are typically on the order of <1 μg per sample. Urine provides for an analysis of soluble

iodine, faecal analysis can be used to assess the fraction of ingested iodine not absorbed by the gut, and tissue is used to assess whole or regional body burdens of iodine.

These methods include arsenious-ceric ion catalytic spectrophotometry, instrumental neutron activation analysis (INAA), inductively coupled plasma atomic emission spectrometry (ICP-AES), and high performance liquid chromatography/ultraviolet-visible detection techniques. The INAA and ICP-AES methods offer the greatest sensitivity for the detection of iodine in human samples (Stephanie, 2014).

A 24-hr urine iodine collection is a useful medical test, an approximately 90% of ingested iodine is excreted in the urine (Stephanie, 2014). For the standardized 24-hr test, a 50 mg iodine load is given first, and 90% of this load is expected to be recovered in the urine of the following 24 hrs. Recovery of less than 90% is taken to mean high retention, i.e. iodine deficiency. In pregnant women the recovery may be less, and on intake, goitrogens can alter test results. If the 24-hr urine collection is not practical, a random urine iodine-to-cretinine ratio can alternatively be used though less reliable.

The available methods include mild acid digestion timed-colorimetric procedure as in Sandell-Kolthoff's Method, the Inductively Coupled Plasma Mass Spectrometry (ICP-MS); a Rapid Test based on iodide-catalyzed oxidation; and Classical titrimetry as well as Random Spot urine iodine concentration. Other methods include thyroid size and serum concentrations of Thyroid Stimulating Hormone (TSH) (Peeters et al., 2010). There are also semi-quantitative methods, microplate method, automated methods, and the

technologically advanced methods include RNAA. Many analytical methods are available for iodine detection and monitoring in human samples. However, some of the methods have the specificity and sensitivity to measure iodine in biological matrices and these methods are approved by WHO (Kharzan et al., 2013). These include Flow Injection Analyser, Intracavity Laser Absorbance, Ion Selective Electrode and Electrochemical Detection.

Controversies in Urinary Iodine Determinations

The available methods for UI determination have been reviewed by Dunn et al., (2004) and concluded that the most practical and simple method involved mild acid digestion and timed colorimetric procedures. This procedure is less time consuming than the more traditional method of dry ashing (WHO/UNICEF/ICCIDD, 2001). Various methods are available for the removal of chromogens and thiocyanates that interfere with the sensitive colorimetry of Sandell-Kolfthoff reaction. In this reaction, acid is first digested under mild conditions and the iodide then determined from its catalytic reduction of cerium ammonium sulphate in the presence of arsenic acid. This method was used in determining the National Health and Nutrition Examination Survey (NHANES) in 1971-1974 and NHANES III in 1988-1994 with populations of 18,617 and 22,070 respectively USA (Soldin, 2002).

UI concentrations determined by ICPMS have a lower limit of detection for the assay (2 μg/L), and the reproducibility of the assay was good. Comparison with the Sandell-Kolthoff reaction method showed a highly significant correlation ($P < 0.000$) and no systematic bias at low iodine concentrations. Chen et al (2005) examined the usefulness of “spot” urine iodine concentrations for populations and concluded that the current data on

UIC as a means of predicting 24-hr urinary iodine excretion (UIE) for estimating population, sodium intake are inadequate and highlight the need for further methodological investigation.

Kharzan et al (2013) reviewed a total of 2030 articles and concluded that the Sandell-Kolthoff reaction method are technically very simple and have been used extensively to measure urinary iodine. The two main methods to determine urinary iodine are the instrumental method and the colorimetric methods in association with Sandell-Kolthoff reaction which has been used by many laboratories to measure levels for many years. Though the classical titration method (CTM) is approved by WHO (Guidelines on Iodine Testings in fluids (blood and urine) WHO 2001, 2004) and used by Gbadebo & Nwufoh, (2010) was neither mentioned by Kharzan et al (2013) nor Soldin (2002) in their reviews.

The CTM is relatively technically simpler, inexpensive, low cost and easier to apply. The limitation, however, is that the urine sample is not digested and determines only iodine from inorganic origin (Gbadegbo & Nwufoh, 2010) as opposed to Sandell-Kolthoff reaction. The Sandell-Kolthoff reaction is subject to the following limitations (WHO A Guide for Programme Managers 2007; Fallouch et al., 2004; Mesquita et al., 2017):

1. The ceric ammonium sulphate (Ce $[NH4]_4(SO_4)_4.2H_2O$) (CAS) reagent generates so much heat, about 150 $^{\circ}C$, during preparation.
2. The procedure requires that tubes be heated for 60 mins at 91-95 ^{o}C temperature. This presupposes that different researchers working at different temperatures within this range could have different results.

3. It is also required to determine the absorbance at either 405 or 420 nm, again the wavelength must be specific.
4. Prior to reading the absorbance, the CAS is supposed to be added 30 mins earlier, and that successive tubes are to be read at the same time intervals as when adding the CAS. Thus procedure is cumbersome and time wasting.
5. The digestion procedure is equally cumbersome, and has no specific end-point.
6. It has been recommended that since the exact temperature, heating time and cooling time may vary, within each assay, the interval between the time of addition of CAS and the time of the reading must be the same for all samples, standards and blank (WHO, A Guide for Programme Managers, 2007). This is also a near – impossibility and that different researchers working at different specifications might have different results.
7. It is also recommended that if different tube sizes are used, corresponding sized holes in the heating block are also used. This is an inconvenience.
8. It is also recommened that it is necessary only heating block should be used and that water, oil or sand bath used is not advisable. This is because the heating block provides uniform and constant heat within the stipulated temperature. This is a restriction on the use of the method.
9. The use of ammonium persulfate as a digestant is equally toxic as chloric acid. It is a mild digesting agent.
10. That digestion is only to eliminate metabolite and contaminants that might interfere with the sensitivity of the automation is not quite true.

11. With regards to the method B where chloric acid is used as a digestant, the explosive nature of the chemical mixture raises a safety concern, and its modification involving the use of stop watch instead of spectrophotometer to measure colour change is not sensitive enough.
12. The procedure is cumbersome, costly, and time consuming for analyzing large sample size.
13. Incapable of releasing all the bound organic iodine due to the use of mild digesting agent, ammonium persulphate. The digesting agent affects the optimal pH and thus reduces the sensitivity of the method.
14. It is a complicated method with extensive measuring time.

In view of the above limitations, a new method, called the Novel method was proposed for urinary iodine analysis in this study. This method is capable of releasing most of the iodine from both organic and inorganic origins due to the use of effective digesting agents, HCl and H_2SO_4. The method determines total iodine in solution. It is cost effective, safer, employs less steps, uses less volumes, less time consuming and easier to use. In this method, the urine sample shall be acid digested at a certain temperature and the liberated iodide ions shall be oxidised with $KMnO_4$ into iodine. Oxalate ions shall then be introduced to reduce the excess $KMnO_4$. The liberated iodine is then analysed by titrating it against thiosulphate solution.

Iodine Deficiency Surveys

WHO recommends that iodine deficiency surveys examine school-age children from 6-12 yrs (WHO/UNICEF/ICCIDD, 2001).

Population coverage, proportion of population and the number of individuals with insufficient iodine intake

The coverage of the estimates for a given WHO region was calculated as the sum of the populations of countries with data divided by the total population of the region. The same procedure was used to calculate global coverage, Table 16.

Table 16: Population Coveragea by TGP Surveys carried out between 1993 and 2003, by UN region

UN region[b]	School-age children (millions)[a]	School-age children surveyed (millions)	Coverage
Africa	154.6	140.5	90.9
Eastern Africa	52.1	46.1	88.4
Middle Africa	19.1	16.4	85.8
Northern Africa	28.7	27.9	97.3
Southern Africa	8.3	7.9	95.4
Western Africa	46.4	42.2	91.1
Asia	521.7	487.4	93.4
Eastern Asia	164.8	148.8	90.3
South-central Asia	246.5	246.5	100.0
South-eastern Asia	79.0	69.9	88.6
Western Asia	31.5	22.2	70.6
Europe	59.1	26.1	44.2
Eastern Europe	25.2	9.2	36.4
Northern Europe	8.6	0.5	5.5
Southern Europe	10.6	9.4	88.2
Western Europe	14.7	7.1	48.4
Latin America and the Caribbean	76.6	50.7	66.2
		1.3	28.4
Caribbean	4.5	16.1	72.0
Central America	22.4	33.3	67.0
South America	49.7		
Northern America	32.4	0.0	0.0
Oceania	3.7	2.9	79.3
Australia-new Zealand	2.3	1.9	81.7
Melanesia	1.3	1.0	80.7
Micronesia	0.040	0.0	0.0
Polynesia	0.054	0.0	0.0
Total	848.0	707.7	83.5

Source: WHO A Guide for Programme Managers, (2007)

National, Regional and Global populations (school-age children and general population) with insufficient iodine intake were estimated based on each country's proportion of population with UI < 100 μg/L. The following method was used:

1. The number of subjects with insufficient iodine intake at the country level was calculated by applying the proportion of population with UI below 100 μg/L to the national population of both children aged 6-12 yrs and general population (all age groups including children aged 6-12 yrs), Table 21. The population figures are based on the year 2002.

   The 95% confidence intervals for the proportion of a population with UI <100 μg/L for each country are presented as a measure of uncertainty.
2. The number of subjects with insufficient iodine intake at the regional level was calculated by summing the number of individuals with UI <100 μg/L in each country of the region and dividing the sum by the total population of all countries with available data. The calculations were made for both WHO and UN regions.
3. The global estimate was calculated by summing the number of individuals with insufficient iodine intake in each region and dividing the sum by the total population of all countries with data available.

The proportion of the population and the number of individuals (school-age children and general population) with insufficient iodine intake (defined as proportion of population with UI < 100 μg/L) by WHO region is presented in Tables 17 and 18; Figure 5.

It is estimated that the iodine intake of 36.5% (285 million) school-age children worldwide is insufficient, Table 17.

Table 17: Proportion of Population, and Number of Individuals with insufficient Iodine Intake in School-Age Children (6-12 yrs), and in the General Population (all age groups) by WHO region, 2003

WHO region[a]	Insufficient iodine intake (UI<100 μg/L)			
	School-age children		General population	
	Proportion (%)	Total number (millions)[b]	Proportion (%)	Total number (millions)[b]
Africa	42.3	49.5	42.6	260.3
Americas	10.1	10.0	9.8	75.1
South-east asia	39.9	95.6	39.8	624.0
Europe	59.9	42.2	56.9	435.5
Eastern Mediterranean	55.4	40.2	54.1	228.5
Western pacific	26.2	48.0	24.0	365.3
Total	36.5	285.4	35.2	1988.7

Source: WHO A Guide for Programme Managers, (2007).

Extrapolating the proportion of school-age children to the general population, it is estimated that nearly two billion individuals have insufficient iodine intake. The most affected region is South-East Asia where 96 million children have a low iodine intake. Africa and the Western Pacific follow, both with an estimated 50 million children with a low iodine intake. Europe and the Eastern Mediterranean harbour about 40 million children each, and the Americas have 10 million. The highest proportions are found in Europe (59.9%) and South-East Asia (39.9%) while the lowest are found in the Americas (10.1%) and the Western Pacific (26.2%).

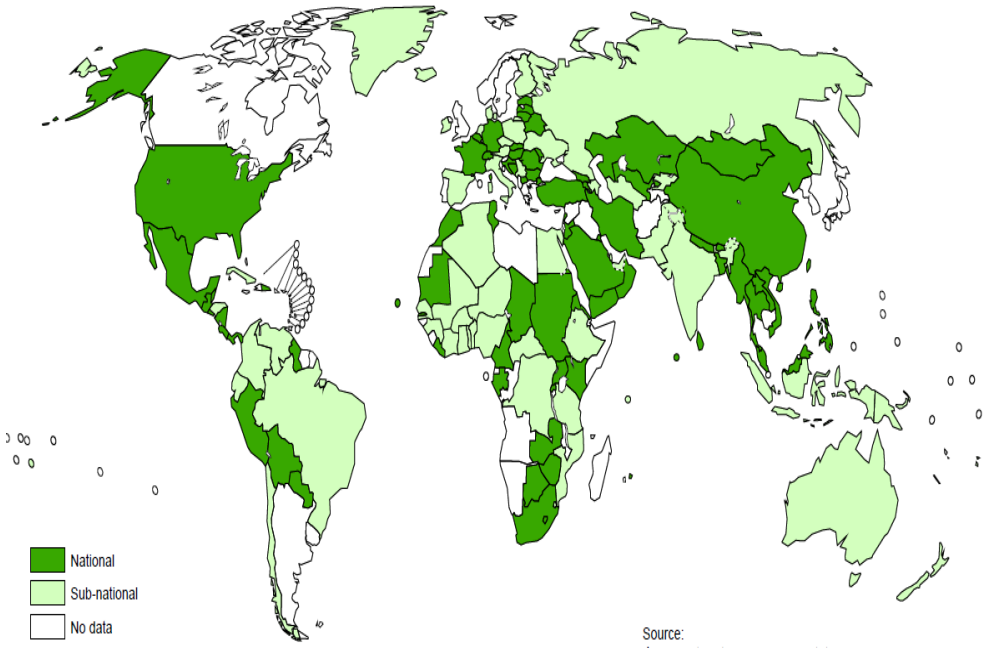


Figure 5: Based on 192 WHO Member States.

Source: Iodine Status Worldwide (2004)

Overall, one third of the world's school-age children population has UI <100 µg/L indicating insufficient iodine intake. This group is therefore exposed to the risk of iodine deficiency. For the six WHO regions the proportion of the population with UI <100 µg/L ranges from 10% (in the Americas) to 60% (in Europe) Table 17.

Noteworthy is the correlation between household coverage of iodised salt and prevalence of low iodine intake. The proportion of households consuming iodized salt increased from 10% in the 1990s (WHO/UNICEF/ICCIDD, 2007) to 66% in the year 2003.

The Americas has the highest number of households consuming iodised salt (90%) and the lowest proportion of its population with an insufficient iodine intake. In contrast, the European Region which has the lowest household consumption of iodised salt (27%), has the highest proportion of its population with an insufficient iodine intake. These results,

however, should not mask the fact that there are large variations both between countries within regions, and within countries themselves. Results of surveys of school-age children were thus extrapolated to the general population, Table 18.

Table 18: Proportion of Population, and Number of Individuals with, insufficient Iodine Intake in School-Age Children (6–12 yrs) and the General Population, by UN region, 2003

	School-age children		General population	
	Prevalence (%)	Total number (millions)[b]	Prevalence (%)	Total number (millions)[b]
Africa	42.7	59.7	43.0	324.2
Eastern Africa	45.1	19.4	45.2	98.2
Middle Africa	32.4	5.1	32.7	26.3
Northern Africa	50.7	14.1	50.6	88.2
Southern Africa	31.6	2.5	31.2	15.4
Western Africa	41.1	18.6	41.4	96.2
Asia	38.3	187.0	35.6	1239.3
Eastern Asia	16.3	24.2	16.3	212.2
South-central Asia	43.2	104.1	41.9	631.9
South-eastern Asia	61.2	46.4	60.5	312.6
Western Asia	53.2	12.2	55.8	82.6
Europe	53.1	26.1	52.7	330.8
Eastern Europe	60.0	15.1	59.9	180.6
Northern Europe	59.3	1.2	59.2	13.0
Southern Europe	47.8	4.1	49.2	58.8
Western Europe	43.6	6.4	42.6	78.5
Latin America and the Caribbean	10.3	7.1	10.0	47.4
Caribbean	69.8	1.7	66.2	13.2
Central America	9.9	2.2	9.7	13.5
South America	7.3	3.3	6.6	20.8
Northern America	9.5	2.8	9.5	27.6
Oceania	59.4	2.1	64.5	19.2
Australia-new Zealand	73.0	1.7	72.8	17.0
Melanesia	32.7	1.4	33.9	2.2
Micronesia	-	-	-	-
Polynesia	-	-	-	-
Total	36.5	285.4	35.2	1988.7

Source: WHO A Guide for Programme Managers, (2007)-Represent No data.

However, it has recently been recognised that national systems to monitor the impact of USI also need to include other vulnerable groups, especially pregnant women. Data for this population group may be considered for future global analysis as more data become available.

Total Goiter Prevalence (TGP) surveys estimated from TGP data from school-age children (6-12 yrs). The worldwide TGP of 15.8% is above the 5% cut-off used to signal a public health problem (WHO/UNICEF/ICCIDD, 2001), Table 19.

Table 19: Type of Total Goitre Prevalence Survey Data by UN Region

UN Region[b]	National	Sub-national	No data
Africa	21	18	14
Eastern Africa	6	5	6
Middle Africa	3	3	3
Northern Africa	3	2	1
Southern Africa	3	1	1
Western Africa	6	7	3
Asia	24	9	14
Eastern Asia	2	0	3
South-central Asia	7	7	0
South-eastern Asia	6	0	5
Western Asia	8	3	6
Europe	9	10	22
Eastern Europe	3	4	3
Northern Europe	0	1	9
Southern Europe	5	3	5
Western Europe	1	2	5
Latin America and the Caribbean	4	3	26
Caribbean	1	0	12
Central America	1	1	6
South America	2	2	8
Northern America	0	0	2
Oceania	0	2	14
Australia-new Zealand	0	1	1
Melanesia	0	1	3
Micronesia	0	0	5
Polynesia	0	0	5
Total	57	43	92

Source: WHO A Guide for Programme Managers, (2007)

Its increase of 31.7% between 1993 and 2003, Table 20, is inconsistent with current iodine status based on UI. Population coverage for TGP surveys is 83.5%, ranging from 46.5% in the Americas to 95.7% in South-East Asia, Table 10. Of the 100 countries with data available on TGP, 57 had nationally representative surveys, covering 43.4% of the school-age children population, Table 20. This has several possible explanations.

Table 20: Total Goitre Prevalence in the General Populationa by UN Region, 2003

UN region[b]	Total goiter prevalence (%)
Africa	26.8
Eastern Africa	29.5
Middle Africa	23.3
Northern Africa	25.3
Southern Africa	29.1
Western Africa	25.9
Asia	14.5
Eastern Asia	5.3
South-central Asia	23.8
South-eastern Asia	8.4
Western Asia	20.4
Europe	16.3
Eastern Europe	27.2
Northern Europe	12.1
Southern Europe	10.9
Western Europe	10.7
Latin America and the Caribbean	4.7
Caribbean	4.9
Central America	8.7
South America	2.9
Northern America	-
Oceania	
Australia-new Zealand	12.9
Melanesia	15.3
Micronesia	4.4
Polynesia	-
	-
Total	15.8

Source: WHO A Guide for Programme Managers, (2007)

First, there is a time lag between the implementation of a salt iodisation programme and the disappearance of clinically detectable goitre (Delange, 2001). This time-lag may be further increased when USI is only partially implemented. Second, 70% of the TGP surveys in the analysis period 1993-2003, Figure 6

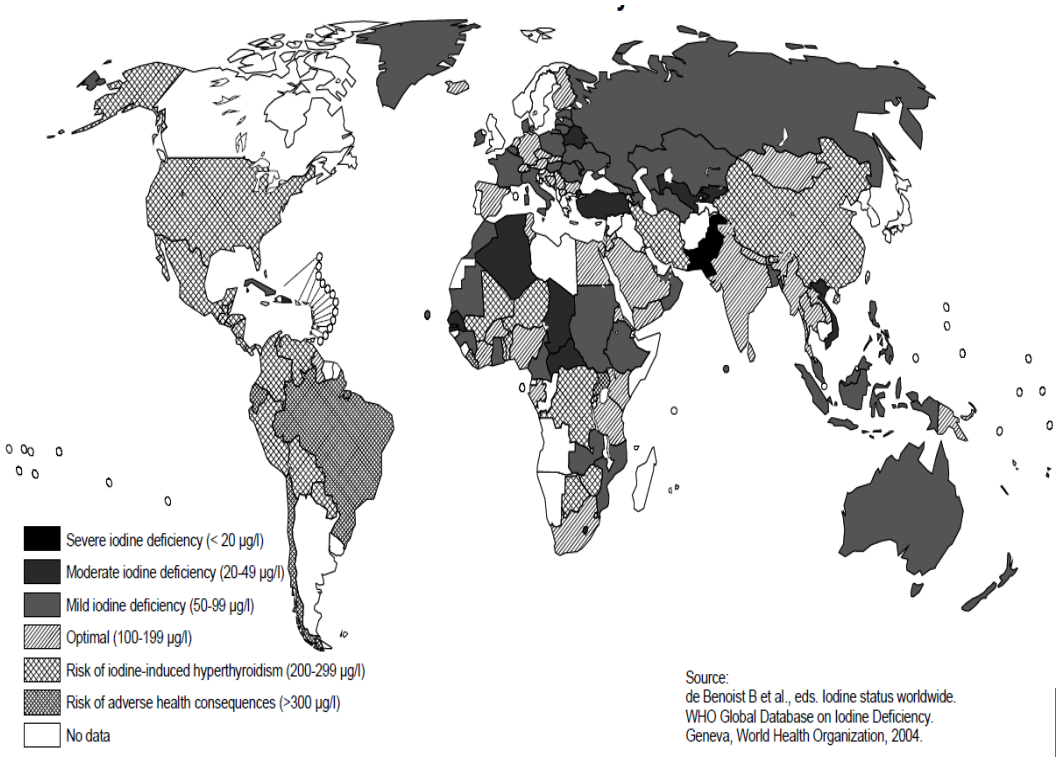


Figure 6: Degree of Public Health Significance of Iodine Nutrition Based on Median Urinary Iodine.
Source: Iodine Status Worldwide, (2004)

WHO Global data based on iodine deficiency Geneva, WHO, 2004

In fact, when analysis is restricted to surveys carried out in the last five years, TGP shows a decrease of 28.9% compared to 1993. Analysis of data available in the WHO database also shows that between 1993 and 1998 TGP was the main indicator used to assess iodine deficiency, while UI was measured only in a few countries. The shift in indicators from TGP to UI over the last decade resulted in less TGP data covering the last five years since many countries only measured UI in their most recent surveys.

Third, in areas affected by mild iodine deficiency, the sensitivity and specificity of TGP measured by palpation are poor (Zimmermann et al., 2000). Ultrasonography is a promising method to overcome the inherent limitations of the clinical assessment of thyroid volume as iodine status improves. New international reference values are now available allowing comparison between countries (Zimmermann et al., 2004). In spite of its limitations for global trend analysis TGP measured by palpation remains a practical indicator for baseline assessment, especially in severely endemic areas (WHO/UNICEF/ICCIDD, 2001). Data on goitre collected between 1993 and 2003 were available from 100 countries, Table 21.

Table 21: Change in Total Goitre Prevalence between 1993 and 2003, by WHO Region

UN region[b]	TGP (%) general population		
	1993	2003	% change
Africa	15.6	28.3	+81.4
Eastern Africa	8.7	4.7	+46.0
Middle Africa	13.0	15.4	+18.5
Northern Africa	11.4	20.6	+80.7
Southern Africa	22.9	37.3	+62.9
Western Africa	9.0	6.1	+32.2
Total	12.0	15.8	+31.7

Source: WHO A Guide for Programme Managers (2007)

Table 22 presents the population coverage for the age group 6-12 yrs based on TGP data by WHO region.

Table 22: Population Coveragea by TGP Surveys carried out between 1993 and 2003, by WHO Region

UN region[b]	School-age children (millions)[c]	School-age children covered (millions)	Coverage (%)
Africa	128.9	117.6	91.2
Eastern Africa	109.0	50.7	46.5
Middle Africa	242.4	232.1	95.7
Northern Africa	81.2	46.9	57.8
Southern Africa	87.1	76.5	87.8
Western Africa	199.4	184.0	92.3
Total	848.0	707.7	83.5

Source: WHO A Guide for Programme Managers, (2007)

Based on population estimates for the year 2002 (UN 2003), Table 23, Figure 7.

Table 23: Type of Total Goitre Prevalence Survey Data by WHO Region

UN region[a]	National	Subnational	No data
Africa	18	17	11
Eastern Africa	4	3	28
Middle Africa	7	1	3
Northern Africa	12	15	25
Southern Africa	10	5	6
Western Africa	6	2	19
Total	57	43	92

Source: WHO A Guide for Programme Managers (2007)

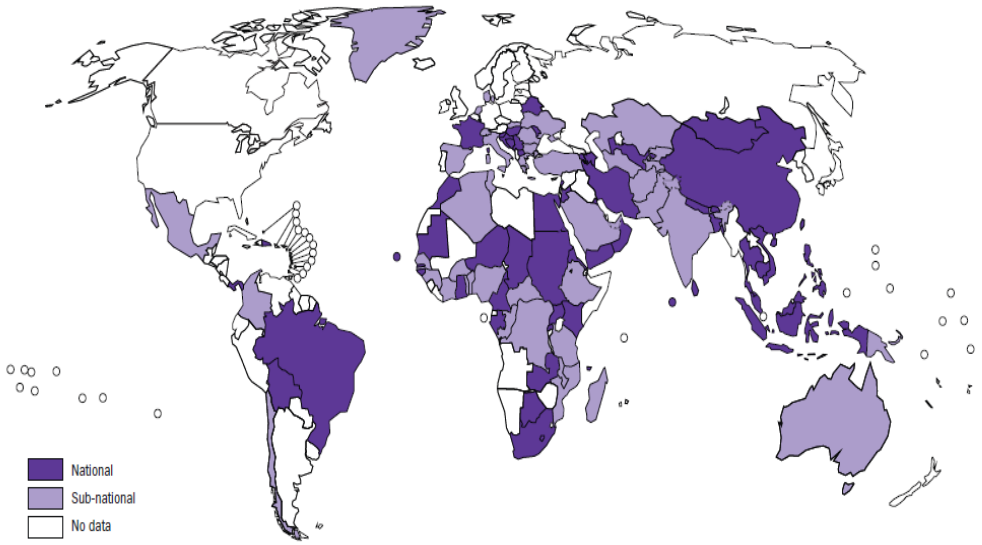


Figure 7: Type of Total Goitre Prevalence Survey

Source: Iodine Status Worldwide (2004).

Table 24 presents the number of countries with national and, if not available, sub-national surveys.

Table 24: Population Coverage by UI Surveys carried out between 1993 and 2003, by WHO Region

UN region[b]	Total number School-age children (millions)[c]	School-age children covered (millions)	Coverage (%)
Africa	128.9	116.9	90.7
Eastern Africa	109.0	968.8	90.6
Middle Africa	242.4	239.4	98.8
Northern	81.2	70.5	86.8
Africa	87.1	72.6	83.4
Southern	199.4	183.0	91.8
Africa			
Western Africa			
Total	848.0	781.2	92.1

Source: WHO A Guide for Programme Managers, (2007)

Figure 8 shows the worldwide coverage of national and sub-national TGP surveys.

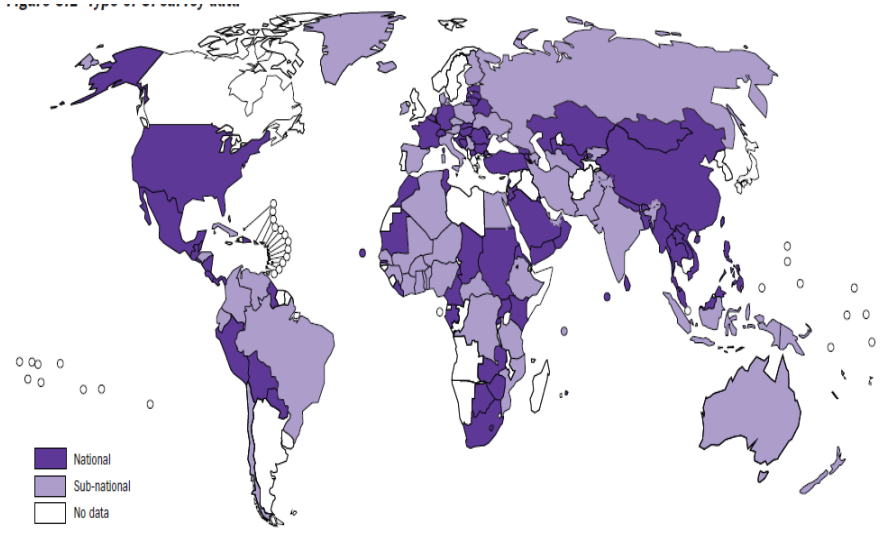


Figure 8: Type of Urinary Iodine Survey Data.

Source: Iodine Status Worldwide, 2004

TGP was computed from data in school-age children. In order to compare present TGP data, with the 1993 TGP estimates which were generated for the general population (WHO/UNICEF/ICC/IDD, 2007). It was necessary to calculate current TGP estimates for the general population. To that end, an algorithm was developed from surveys available in the database that measured prevalence in both population groups. Eight countries were found to have carried out such surveys between 1993 and 2003: Burkina Faso, Ethiopia, France (the island of Réunion), Guinea-Bissau, India, Islamic Republic of Iran, Italy and the Philippines.

A total of 23 pairs of points corresponding to different population subgroups were included. Goitre prevalences by country, region (both WHO and UN) and worldwide were derived for the general population, applying the algorithm described above at country level, along with the point estimates of

TGP, 95% confidence intervals of TGP for each country are presented as a measure of uncertainty, Table 22.

TGP was computed from data in school-age children. In order to compare

Urinary Iodine (UI) Surveys

Data on UI collected between 1993 and 2003 were available from 126 countries. Sixty-six countries have no data on UI, Table 25, Figure 9.

Table 25: Type of Urinary Iodide (UI) survey data by WHO region

UN region[a]	National	Sub-national	No data
Africa	17	17	12
Eastern Africa	13	7	15
Middle Africa	7	2	2
Northern Africa	21	17	14
Southern Africa	11	4	6
Western Africa	6	4	17
Total	75	51	66

Source: WHO A Guide for programme Managers, (2007)

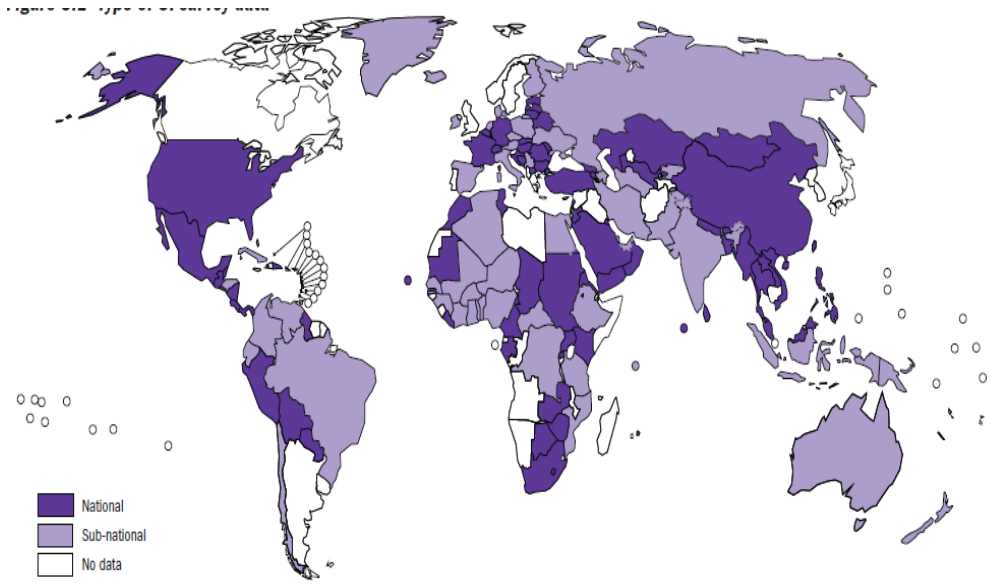


Figure 9: Type of Urinary Iodine Survey Data.

Source**:** Iodine Status Worldwide, (2004)

Overall, the available UI data covers 92.1% of the world's 6-12 yr old population. Regional population coverage varies from 83.4% in the Eastern Mediterranean to 98.8% in South-East Asia. Of the 126 countries with data available on UI, 75 have nationally representative surveys covering 45.7% of the school-age children population.

Classification of countries by degree of Public Health significance of iodine nutrition based on Median UI

Iodine nutrition is optimal in 43 countries as shown in Table 26.

Table 26: Number of Countries Classified by Degrees of Public Health Significance of Iodine Nutrition Based on Median UI in School-Age Children, by UN region, 2003

UN region[a]	Severe iodine deficiency (median UI <20 μ/l)	Moderate iodine deficiency (median UI 20-49 μ/l)	Mild iodine deficiency (median UI 59-99 μ/l)	Optimal iodine deficiency (median UI 100-199 μ/l)	Risk of IIH in susceptible groups (median UI 200-299 μ/l)	Risk of adverse health consequences (median UI ≥300 μ/l)	No data
Africa	0	6	10	13	7	2	15
Eastern Africa	0	0	3	4	2	1	7
Middle Africa	0	2	1	1	1	0	4
Northern Africa	0	1	2	2	0	0	1
Southern Africa	0	1	0	2	1	0	1
Western Africa	0	2	4	4	3	1	2
Asia	1	4	12	12	5	0	13
Eastern Asia	0	0	0	1	1	0	3
South-central Asia	1	2	4	3	2	0	2
South-eastern Asia	0	1	3	3	0	0	4
Western Asia	0	1	5	5	2	0	4
Caribbean	0	0	1	2	6	0	0
Central America	0	0	1	1	5	3	3
South America	0	0	0	0	0	0	0
Northern America	0	0	0	1	0	0	1
Oceania	0	1	2	1	0	0	12

Table 26: continued

Australia-new Zealand	0	0	2	0	0	0	0
Melanesia	0	1	0	1	0	0	2
Micronesia	0	0	0	0	0	0	5
Polynesia	0	0	0	0	0	0	5
Total	1	13	40	43	24	5	66

Source: WHO A Guide for Programme Managers, (2007)

The number of countries with iodine deficiency as a public health problem decreased from 110 to 54 between 1993 (using TGP as an indicator, Table 23) and 2003 using UI (Table 25). Nevertheless, in 54 countries, located in all regions of the world, the iodine intake of the population is insufficient and iodine deficiency with its impact on health and development is still a public health concern. In these countries USI needs to be strengthened and fully implemented. Iodine intake is more than adequate, with a median UI between 200 and 299 μg/L in 24 countries. Here attention should be drawn to the emerging risk of iodine-induced hyperthyroidism in susceptible groups following introduction of iodised salt. Five countries have a median UI equal to or above 300 μg/L indicating an excessive iodine intake and are therefore exposed to the risk of hyperthyroidism and iodine toxicity, Table 27.

Table 27: Number of Countries Classified by Degrees of Public Health Significance of Iodine Nutrition Based on Median UI in School-Age Children by WHO region, 2003

WHO region[a]	Severe iodine deficiency (median UI <20 μ/I)	Moderate iodine deficiency (median UI 20-49 μ/I)	Mild iodine deficiency (median UI 59-99μ/I)	Optimal iodine deficiency (median UI 100-199 μ/I)	Risk of IIH in susceptible groups (median UI 200-299 μ/I)	Risk of adverse health consequences (median UI ≥300 μ/I)	No data
Africa	0	6	8	11	7	2	12
Eastern Africa	0	1	1	3	12	3	15
Middle Africa	0	0	3	5	1	0	2
Northern Africa	0	4	19	15	0	0	14
Southern Africa	1	0	5	6	3	0	6
Western Africa	0	2	4	3	1	0	17
Total	1	13	40	43	24	5	66

Source: WHO A Guide for Programme Managers, (2007)

Elevated median UI is most likely due to high levels of iodine added to salt. Salt quality monitoring should be re-inforced to ensure that the level of salt fortification with iodine is not too high but is adequate to ensure optimal iodine nutrition. Sixty-six countries have no data on UI and iodine nutrition can therefore not be classified. Even though iodine deficiency is unlikely in many of these countries, urinary iodine surveys should be performed in order to investigate the level of iodine intake and evaluate the effectiveness of pre-existing salt iodisation programmes. There is evidence that iodine deficiency may be re-emerging in countries that were previously thoμght to be iodine sufficient, like Australia and New Zealand.

Goitre prevalence in the general population is presented with the purpose of comparing the current estimate with that of 1993. Globally, the TGP in the general population is estimated to be 15.8%, varying between 4.7% in the Americas to 28.3% in Africa. When comparing current TGP estimates with the 1993 estimates, TGP has increased by 31.7% worldwide. This masks a decrease in two regions of 46.0% in the Americas and 32.2% in the Western Pacific. All other regions experienced an increase in TGP ranging from 18.5% in South-East Asia to 81.4% in Africa.

In 54 countries the population has insufficient iodine intake as indicated by a median UI < 100 μg/L. These countries are classified as iodine deficient: one country is severely deficient, 13 are moderately deficient and 40 mildly deficient, Table 28. In 43 countries, the population have adequate iodine intake with a median UI between 100 and 199 μg/L. Iodine nutrition of these countries is considered as optimal. In 24 countries, median UI is between

200 and 299 μg/L indicating that the population has more than adequate iodine intake.

In these countries, there is a risk of iodine-induced hyperthyroidism in susceptible groups. In 5 countries, there is excessive iodine intake as shown by a median UI >300 μg/L, Table 27. In these countries, there is a risk of iodine-induced hyperthyroidism and other adverse health consequences. Data gathered in the WHO Global Database on Iodine Deficiency permit a description to be made of the magnitude, severity and distribution of iodine deficiency worldwide and facilitates decisions on the most effective strategy to eliminate iodine deficiency .The remaining 66 countries, Table 27, lacking data, or lacking recent data, represent only 7.9% of the world's school-age population. However, the risk of iodine deficiency is unlikely to be a public health problem in many of these countries.

Groups at Risk of Iodine Deficiency

Historically, iodine deficiency was endemic in mountainous regions of the US and Mexico and in the "goiter belt" great lakes, but now iodated salt policy and other factors have greatly reduced this canker. Worldwide, however, iodine deficiency remains a public threat to health in 47 countries (Zimmerman, 2008), and about 2.2 billion people (38% of the world's population) live in areas with iodine deficiency (ICCIDD, 2010). International efforts since the early 1990s have dramatically reduced the incidence of IDD, but some groups of people are still at risk of inadequate iodine intake. These groups of people include those living in regions with iodine deficient soils, e.g. the Himalayas, Alps and Andes Regions, and river valleys prone to flooding, especially in South and Southeast Asia (Zimmerman, 2008), the

second group are people with marginal iodine status who eat foods containing goitrogens, these are substances that interfere with the uptake of iodine in the thyroid, and can exacerbate iodine deficiency (Institute of Medicine, 2001). Foods high in goitrogens include soy, cassava, cabbage, broccoli, cauliflower, and other cruciferous vegetables. Deficiency iron and/or vitamin A may also be goitrogenic (Hess, 2010), for most people who have adequate iodine intakes and eat a variety of foods this is not a concern. The third group consists of people living in regions with iodine-deficient soils; such populations are at a risk of iodine deficiency unless they have access to iodised salt or foods produced outside the iodine-deficient area.

People who do not use iodised salt comprises the fourth group: currently about 70% of households worldwide use iodised salt but iodine insufficiency is still prevalent in certain regions. In Europe 52% has insufficient intake, and only about 49% of households in Europe have access to iodised salt according UNICEF reports. Iodine insufficiency is also prevalent in Africa, Southeast Asia and the Eastern Mediterranean have rates of iodised salt use range from 47-67% (WHO/UNICEF/ICCIDD, 2007). Worldwide it is estimated that about 31% of school-age children do not have access to iodised salt (Anderson et al., 2010). The last group is made up of pregnant women, during pregnancy, the RDA for iodine increase from 150-220 μg/dy (Institute of Medicine, 2001).

Iodine Risk Assessment

WHO considers iodine deficiency as a world wide public health risk. In Ghana, the first baseline survey conducted in 1991 and 1994 in 27 districts to ascertain the risk of iodine deficiency revealed varying degrees of

endemicity ranged from mild to severe Total Goitre Rate (Buxton & Baguuna, (2012). Buxton & Baguuna (2012) indicated that a reanalysis of the 2007 baseline data suggested that a 51.8% rather than 33.3% of Ghana's 1,194 districts have been afflicted with IDD deserving public health attention vis-à-vis the estimated 50% of households that use iodised salt in Ghana (WHO/UNICEF/ICCIDD, 2001; 2004).

Moreover, the medium term health strategy for Ghana towards vision 2010 and revised in August 2000 still maintained that levels of IDDs were high in the Northern parts and some parts of the Western Region according to Buxton & Baguuna (2012). The population in these IDD endemic areas faces the risk of hypothyroidism, high school drop-out rate, underutilization of educational opportunities, and that handicapped children may not be able to cope on their own. The population could also be deprived of their productive and reproductive contributions (Buxton & Baguuna, 2012). IDD differs in spectrum across all ages. The foetus suffers congenital anomalies and perinatal mortality; endemic cretinism and mental deficiency are the bane of the neonate.

Children and adolescents are proned to impaired mental function, and delayed physical development whilst the adult grapples with impaired mental function and iodine-induced hyperthyroidism. This was revealed by a risk assessment study on dietary intake in Honk Kong adults and severe cognitive disability and death (WHO, Effect and Safety of Salt Iodisation to prevent IDD: a systematic review with meta-analyses, 2014). A health risk assessment (also referred to as a health appraisal and health and well-being assessment) is one of the most widely used screening tools in the field of health promotion

and is often the first step in multi-component health promotion programs. Risk assessment is the determination of quantitative or qualitative estimate of risk related to a well-defined situation and a recognized threat (also called hazard). FAO/WHO definition is the identification, evaluation and estimation of the levels of risks involved in a situation, their comparison against benchmarks or standards, and determination of an acceptable level of risk. In the food industry, it consists of hazard identification, hazard characterization, exposure assessment and risk characterization. Quantitative risk assessment requires calculations of two components of risk (R): the magnitude of potential loss (L), and the probability (P) that the loss will occur. An acceptable risk is one that is understood and tolerated usually because the cost or difficulty of implementing an effective countermeasure for the associated vulnerability exceeds the expectation of the loss (L). "Health risk assessment" includes variations, such as risk as the type and severity of response, with or without a probabilistic context.For public health and environmental decisions, loss is simply a verbal description of the outcome, such as increased cancer incidence or incidence of birth defects. In that case, the "risk" is expressed as

$$Ri = p\,(Li) \qquad (3)$$

I_2 risk estimate considers information on the number of individuals exposed, it is termed a "population risk" and it is the units of expected increased cases per a time period. I_2 risk estimate does not take into account the number of individuals exposed, it is termed an "individual risk" and is in units of incidence rate per a time period. Populations risks are of more use for cost/benefit analysis, individual risk are of more use for evaluating whether risks to individuals are susceptible. In the context of public health, risk

assessment is the process of characterizing the nature and likelihood of a harmful effect to individuals or populations from certain human activities. Health risk assessment can be mostly qualitative or can include statistical estimates of probabilities for specific populations. In Environmental Risk Assessment (ERA), for example, the undesired event is usually detrimental to organisms, populations or ecosystems. The current ERAs usually compare an exposure to a no-effect level, such as the Predicted Environmental Concentration (PEC/PNEC) ratio. Although this type of ratio is useful and often used in regulation purposes, it is only an indication of an exceeded apparent threshold.

Iodine Deficiency Disorders in Ghana

Before 1990, few countries were iodine sufficient globally. Between February, 2012 and December, 2014, 19 countries have changed their iodine status. Eight countries previously classified as mildly or moderately deficient have now reached sufficient iodine nutrition at the national level. These include Afghanistan, Australia, Ghana, and Guatemala. In Ghana, 120,000 children born each year are at risk of intellectual impairment because of iodine deficiency (ID). Approximately 30% of these babies are severely impaired and suffer improper development, mental illness, and reduced intelligence (Buxton & Baguuna, 2012). The first baseline survey in Ghana on IDD, Table 42, was conducted in 1991 and 1994 in 27 districts, varying degrees of endemicity was found which ranged from mild to severe Total Goiter Rate (TGR) (ICCIDD, 2008; Buxton & Baguuna, 2012). In 2001, MUIC was 77 μg/L to 183 μg/L and another study among school children revealed a MUIC of 67.9 μg/L (Amoah et al., 2004). Yet another study in two districts in UE region in 2007

showed a drop in TGR but MUIC was 51.6 μg/L in Jirapa and Bongo respectively, these values were below the satisfactory threshold of 100 μg/L.

It also revealed that 48.5 and 36.3% of households in Jirapa and Bongo districts respectively used iodised salt adequately. The same survey showed that 58% of salt sold in the markets was iodised, yet below 20 ppm, compared with the mandated iodisation level of 25-50 ppm (Buxton & Baguuna, 2012). A re-analysis of the 2007 baseline data suggested a 51.8% rather than 33.3% of Ghana's 1,194 districts were afflicted with IDD, deserving public health attention (Buxton & Baguuna, 2012).

In another study in the Northern parts of Ghana, a 68.8% of 1,061 subjects had goiter, and MUIC was 1.6 μg. However, it has been estimated that approximately 50% of households use iodised salt in Ghana (WHO/UNICEF/ICCIDD, 2001; UNICEF, 2004). The medium term health strategy for Ghana towards vision 2000 revised in August 2000 still maintained that levels of IDDs were high in the Northern parts and some parts of the Western Region (Ghana Health Service, 2000; Buxton & Baguuna, 2012). In the Western region of Ghana household utilization of iodised salt was 53.2, 67. 5, and 78.1% respectively in 2003, 2005 and 2007; and that 51.7% households in Bia District in 2003 had their consumption rose to 76.7 and 77.4% in 2005 and 2007 respectively (Buxton & Baguuna, 2012). Findings of the 2007 survey further revealed that the TGR stood at 18.8% which was quite high (Buxton & Baguuna, 2012). Buxton & Baguuna (2012) also revealed that 75.6% of households in the Bia District of Western Region consumed iodised salt and the knowledge of iodised salt quite high between 64-72.0%. They concluded that only 64.6% exclusively used iodised salt, this

did not necessarily translate into an increase in the number of households which used iodised salt.

Violating the law under Food and Drugs Amended Act attract a fine or imprisonment not exceeding 2 years or both (Ghana Health Service, 2015). Asibey-Berko et al., (1995) indicated that 33% of 30 districts in Ghana had severe IDD. Awareness level was 80%, but knowledge on goiter was 20%; prevalence increased from 8.1% in 1998 to 67% in 2002. (Asibey-Berko et al, 2002).

The first national survey was conducted from 1991-1994. The survey established that 33% of districts had significant iodine deficiency, providing for the mandatory fortification of salt **(**National Iodine Survey Report GHANA 2015). In 1996, Ghana adopted the USI programme and then passed a law to compulsorily iodise edible salt in the country. In 2010, another survey showed an increase in IDD. The recent survey showed adequacy in national iodine intake (Nationa Iodine Survey Report GHANA 2015), yet Sarfo-Katanka et al., (2017) and Aryee et al., (2018) reported an increase in IDD in the Central region of Ghana.

The Mandatory Iodised Salt Law in Ghana

Act 1992 PNDCL 305B Section 6:

"A person shall not mine salt for human/animal consumption, import, manufacture, package, label, advertise, store, deliver, distribute, trade, sell or export any salt, that is not fortified with KIO_3 in accordance with this Act".

Salt is fortified where it has additive such as KIO_3 or other nutritional substance added to it to enhance its nutritional value. The Ghana Standard

Board (GSB) shall determine and publish in the gazette and newspapers nationwide the standard for the fortification of salt under this Act.

Food and Drugs Act Section 6: Regulation of iodised salt

The law was passed in 1996 for USI in Ghana. The mandatory level initially was 100 ppm and then revised to 25-50 ppm. According to GS 154:2006, that is, Standard for Salt Fortified with Iodine shall contain not less than 50 ppm at the production point and not less than 25 ppm at retail level. The iodisation of salt is mandatory and the Nutrition Unit of Ghana Health Service's function is both coordination and monitoring. It is the Government agency to address IDD. Provisions are made in the Guidelines for Market Surveillance in the handling of non-conforming goods including salt. Sanctions include seizure, administrative charges, and supervising re-iodisation at a fee (Regulation of Iodised Salt, FDA).

Chapter Summary

Iodine is required by the thyroid gland to produce hormones for the body's metabolism. The lack of iodine leads to certain diseases called iodine deficiency disorders (IDD). Notable among these are goiter and cretinism.

Iodised salt consumption was adopted as a dietary preventive measure to eliminate this disorder through a programme called universal salt iodisation (USI). In 1996 Ghana adopted this programme and passed a law to make iodisation of salt mandatory. Prior to this Ghana conducted a baseline survey on iodine deficiency which showed varying degrees of endemicity. Recent survey in 2015 however showed adequacy in national iodine intake, yet Sarfo-Katanka et al.,(2017) and Aryee et al.,(2018) reported an increase in the disorder in the Central region of Ghana.

CHAPTER THREE

RESEARCH METHODS

Introduction

Effective methods for analyzing urinary iodine and determining the iodine status of population is one of the primary focus of this study.

Iodine Deficiency Disorder (IDD) is the single most preventable disease (Stephanie, 2014). Its prevention is primarily by dietary means through the consumption of iodated salt (and or other means prescribed by a certified health practitioner). IDD has been declared by WHO as a public health problem due to its devastating effect on the health of fetus, neonates, children and adult. This deficiency results in the negative productivity of the economy and development of countries.

Ghana legislated in 1996 by Act of Parliament to mandate that all edible salts meant for sale and consumption should be iodised to the required level of iodine. This law was necessitated when a National Survey conducted by Ghana Government revealed that the iodine status of the population was inadequate, and that there was the need to correct this defect by a law. However, after 20 years of promulgation of this law, IDD still persists in certain parts of Ghana (Sarfo-Kantanka et al., 2017). This study therefore finds the levels of iodine in iodisd salt and in urine to predict reasons for the prevalence in Central region of Ghana.

This chapter shall cover the research design, the study area, and the study populations. It shall also consider the sampling procedures and data collection instruments, procedures and analysis of the samples.

The Research Design

Justification of the Design

The research approach adopted is a blend of descriptive, experimental and fixed designs. It is descriptive because questionnaire was administered to gather information about the pattern of behaviour of the respondents (descriptive) towards eliminating IDD in Ghana. The questionnaire specifically sought information about their feeding habits, their knowledge about iodated salt, the mandatory iodated salt law, and their iodine status. The data obtained was resolved quantitatively using SPSS and Excel to access the interrelationships of the predictors and iodine deficiency in the study population.

The research design could also be described as experimental, because salt and urine samples were collected and analysed chemically in the laboratory for their iodine concentration to obtain an empirical data. These two designs (descriptive and experimental) altogether constitute a fixed design because the variables were measured quantitatively to obtain empirical data for assessing the cause of prevalence of IDD in Ghana.

The Study Area and Location

Figure 10 shows the map of Cape Coast Metropolis and the Agona West Municipality in the Central Region of Ghana where urine samples were collected. In the Cape Coast Metropolis the locations were Calvary Hillcrest Schools, and the University of Cape Coast, all at the North sub Metro. In the Agona West Municipality the samples were collected at Agona Swedru.

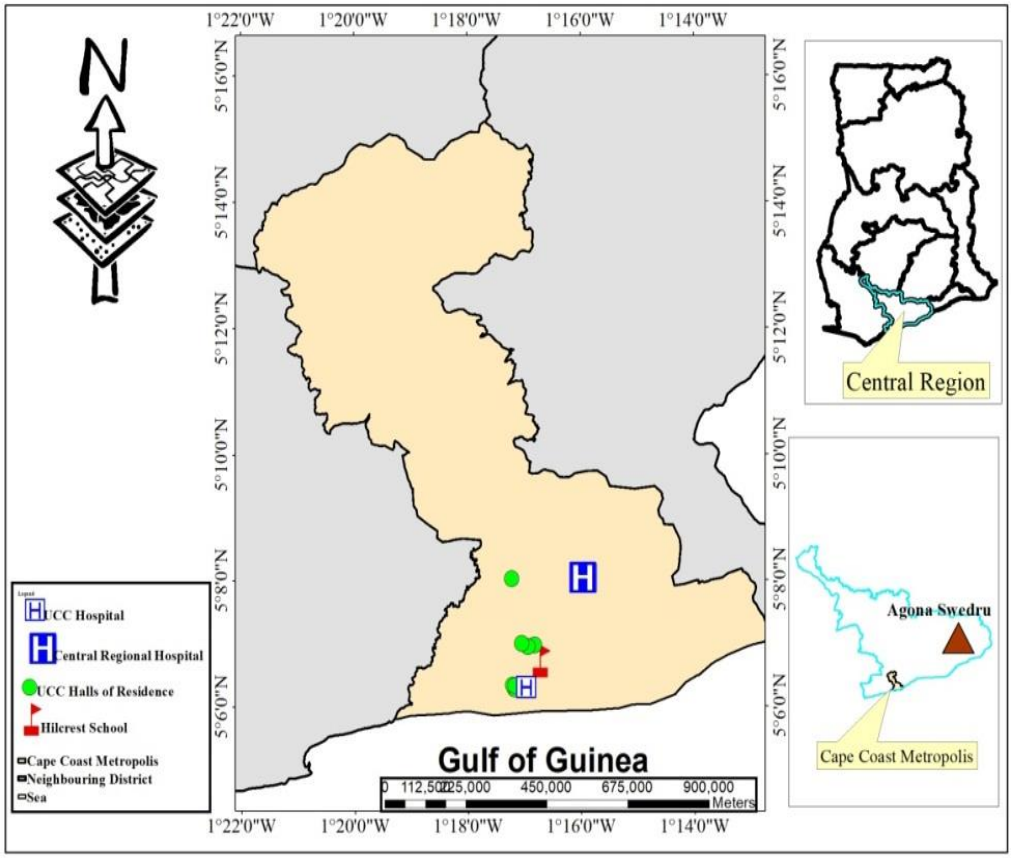


Figure 10: Map of Cape Coast Showing Points of Urine Collection.

Source: Geography Department, University of Cape Coast, Ghana

The study was carried out in an eight step approach, Figure 11.

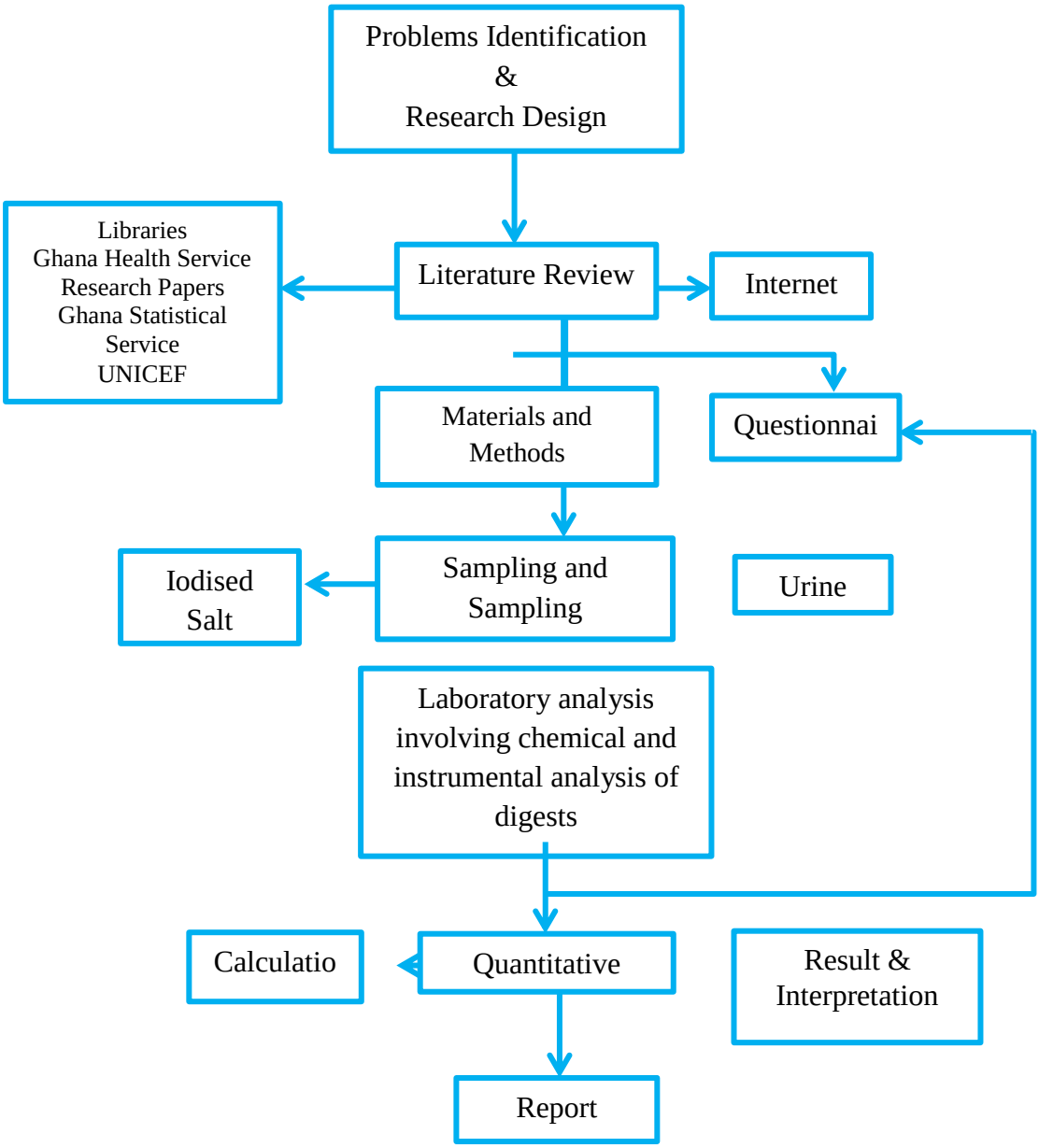


Figure 11: Flow Chart of the Research Approach.

The first step involved the identification of the problem in the research area through planned visits to the selected sites to interact with participants to explain to them the purpose of the research. The second step is about literature search for information about the research area, the research topic and previous work that have been done. The third step included gathering of all the necessary materials required for the sampling and analysis. Administering of

questionnaire to participants constituted the fourth step. The fifth step involved sampling, sample preparation at the research area and at the laboratory respectively. The sixth step was the determination of the iodine in the various samples at the laboratory. The seventh step was the quantitative analysis of the data generated by the titrimetric, spectrophotometric and questionnaire analysis. The last step is the writing of the report.

Target Population for Questionnaire

Two hundred and fifty (250) respondents answered the questionnaire. It was characterized by biosocial and sociocultural indicators such as sex, age, education and other predictors. The respondents were broadly categorized as goitrous and non-goitrous.

The goitrous includes patients who have been diagnosed with thyroid disorders. The non-goitrous category largely comprised of individuals who have not been diagnosed. They were classified as Academic and Residential. The academic is subdivided into tertiary students, pupils and staff (of a preparatory school).

The Study Population for Urine Sampling

The total population for the urine study was 1,048 individuals.

These included members of staff and pupils of Calvary Hillcrest Schools in Cape Coast, the undergraduates of University of Cape Coast 2016/2017 academic year; and a household in Agona Swedru, in the Agona West District of Central Region of Ghana. All the populations were resident in the Central Region of Ghana.

Sampling Procedures

Urine Sample Collection

One thousand and fourty-eight (1,048) human urine samples were obtained with the help of the University of Cape Coast Hospital Staff. Prior to the collection, the consent of the volunteers was sought for after explaining to them the details of this research, confidentiality of the participants and of the results; and the rights of participants to withdraw as spelt out in the consent document granted by the Institutional Review Board (IRB) of UCC. The urine samples were collected in seven months, from June 2016 to December 2016. Each volunteer was given a sterilized vial and a pair of hand gloves and an envelope by the hospital staff. They were directed as to how to fill the vial with one's own urine without contamination. Volunteers were also advised to conceal the urine in the envelope provided.

The vials were labelled with code numbers and not the names of the participants. Having collected the urine sample, it is registered and placed in ice chest and transported to the laboratory. It must be emphasized that urine samples of patients, and market volunteers could not be obtained.

Iodised Salt Sampling

Packaged and unpackaged iodised salt samples were obtained from the open markets and supermarket at random. The packaged salts were in a Low Density Polythylene (LDPE) bags and labelled according to the manufacturers' specifications. The unpackaged salt was sold in trays, and did not have labeling. The salt types were cooking and table salt. They were also coded and sent to the laboratory for analysis. The total number of salt samples was 126.

The salt samples were collected from selected towns, market centres and supermarkets across Ghana. The markets in the Greater Accra Region included Agbogbloshie, Malata, Kaneshie, Makola, Madina, Nima, Ashiaman, Tema, Sege, and Kasei. Ho, Aflao, Blekusu, Adina, Afiadenyigba, Anloga and Dabala constitute the markets in the Volta region. The other towns and markets are Tamale in the Northern region, Wa in the Upper West region, Bolgatanga in the Upper East region and Sunyani in the Brong Ahafo region. The rest were market centres in Koforidua, Krobo – Odumase and Akim Oda in the Eastern region. In the Ashanti region the samples were picked from Kejetia market in Kumasi. The Kotokuraba market in Cape Coast and Kasoa market in Kasoa made up the towns in Central region whilst Takoradi, Axim and Half- Assini markets constitute the towns in Western region. The reasons for such wide coverage included sample size, the density of the population, the economic activity of the town, the availability and the distribution of the iodised salt.

Laboratory Analysis of Samples

Reagents and Chemicals

Sodium sulphite, $Na_2S_2O_3$ (99.9%); Concentrated sulphuric acid, H_2SO_4 (98.0%); Potassium iodide, KI(s), (99.9%); Starch, $C_6H_{10}O_5$(s) (99.9%); Concentrated hydrochlric acid, HCl, (97.0%); Concentrated nitric acid, HNO_3,(68.0%); Ammonium chloride, NH_4Cl(s), (99.9%); Phenolphthalin, $C_{20}H_{14}O_4$(s) (98.0%); Ammonium oxalate, $NH_4C_2O_6$,(99.9%); Eriochrome Black T, $C_{20}H_{12}N_3O_7SN_a$(s),(99.0%); Ethylene diammine tetraacetic acid, $C_{10}H_{16}N_2O_8$(s);(99.9%); Calcium carbonate, $CaCO_3$(s),(99.9%); 100 ppm Pb^{2+} (aq), Agilent Standard Mix,

USA,(99.9%);100 ppm Cd^{2+} (aq), Agilent Standard Mix, USA,(99.9%); 100 ppm Fe^{2+} (aq), Agilent Standard Mix, USA,(99.9%); 100 ppm Zn^{2+} (aq), Agilent Standard Mix, USA,(99.9%); 100 ppm Al^{3+} (aq), Agilent Standard Mix, USA,(99.9%); Concentrated hydrogen peroxide, H_2O_2,(35.0%); Potassium iodate, KIO_3(s), (99.9%); Minimum recovery diluent,(99.9%); Potato dextrose agar,(99.9%);

Eosin methylene blue agar,(99.9%);

Amoxycillin capsules, (99.9%); Ethanol, C_2H_5OH, (99.9%); Sodium chloride, NaCl(s), (99.9%); Ammonium persulphate, $H_8N_2O_8S_2$(s), (99.9%); Arsenic trioxide, AS_2O_3(s), (99.9%); Sodium hydroxide, NaOH(s), (99.9%);

Cerric ammonium sulphate, $Ce(NH_4)_4(SO_4)4.2H_2O$(s), (99.9%); Deionized water; Sodium thiosulphate, $Na_2S_2O_3$(s), (99.9%); Potassium permanganate, $KMnO_4$(s),(98.0%); Potassium oxalate, $K_2C_2O_4$(s), (99.0%); Detergent.

Equipment

UV – VIS spectrophotometer, UV-Mini 1240, Shimadzu, Japan

Atomic Absorption Spectrophotometer, AA-7,000, Shimadzu, Japan

Refrigerator, ZANUSSI, FREEZONE, England

Combined Hotplate and Magnetic Stirrer, mLw 12H3, Germany

Waterbath, Yamato Scientific, HD 42N, Japan

Electric Oven (with in-built thermostat), Ecocell, MMM Medcenter, England

Cuvettes, path length of 1 cm

Preparation of Reagents and Solutions

0.01M $Na_2S_2O_{3\,(aq)}$

Sodium sulphite pentahydrate, $Na_2S_2O_35H_2O$, 1.240 g was weighed into I L volumetric flask. It was dissolved with deionized water, and topped to the mark, and stored in a cool, dark place.

1.0M $Na_2SO_{4\,(aq)}$

Concentrated $Na_2SO_{4(aq)}$ (6 mL) was slowly added to 90 mL deionized water in a 100 mL in a beaker, and then made to 100 mL.

10% $KI_{\,(aq)}$

Potassium Iodide $(KI)_{\,(s)}$, 10.000 g was weighed into 100 mL volumetric flask, and dissolved with deionized water to the mark. The solution was then transferred into a brown bottle and stored in a cool, dark place.

Starch indicator solution

Sodium chloride, $NaCl_{(s)}$ 15.000 g was dissolved in 100 mL beaker. The beaker was heated until excess salt dissolved. The solution was cooled and decanted. Then 1.000 g of starch was also weighed into 50 mL beaker, deionized water was added to form a suspension, it was boiled until it completely dissolved. The saturated $NaCl_{(aq)}$ was added to make 100 mL starch solution.

10% $NH_4Cl_{\,(aq)}$

Analytical grade $NH_4Cl_{(s)}$, 10.0000 g was dissolved in 1000 mL of deionized water.

Phenolphthalein indicator

Powdered phenolphthalein, 0.1001 g was dissolved in 50 mL ethanol and 50 mL deionized water. The resulting solution was stirred constantly and filtered.

10% Ammonium oxalate solution

Ammonium oxalate monohydrate, 10.0000 g was dissolved in 100 mL of deionized water.

10% NH_3-NH_4Cl buffer, pH 10

Analytical grade $NH_4Cl_{(s)}$, 3.000 g was dissolved in 100 mL deionized water. 285 mL of concentrated NH_3 solution was added and the resulting solution diluted to 500 mL with deionized water.

Eriochrome Black T indicator

Eriochrome Black T powder, 0.250 g was dissolved in 50 mL of industrial spirit.

0.01M Ethylene diammine tetraacetic acid (EDTA)

EDTA, 5.001 g was dissolved in deionized water and diluted to 1L in a 1L volumetric flask and then standardised against standard calcium solution.

100 µg/L $Pb^{2+}_{(aq)}$

Twenty-five milliters (25mL) of 1000 ppm $Pb^{2+}_{(aq)}$ was measured into a 250 mL volumetric flask, and topped to the mark with deionized water. This gives a stock solution of 100 ppm, working standards of 10, 20, 30, 40, 50, and 60 ppm were prepared from this stock solution of 100 ppm.

100 µg/L $Cd^{2+}_{(aq)}$

Twenty-five milliters (25mL) of 1000 ppm $Cd^{2+}_{(aq)}$ was measured into a 250 mL volumetric flask, and topped to the mark. This gives a stock solution of 100 ppm, working standards of 10, 20, 30, 40, 50, and 60 ppm were prepared from this stock solution of 100 ppm.

100 µg/L $Fe^{2+}_{(aq)}$

Twenty-five milliters (25mL) of 1000 ppm $Fe^{2+}_{(aq)}$ was measured into a 250 mL volumetric flask, and topped to the mark with deionized water. This gives

a stock solution of 100 ppm, working standards of 10, 20, 30, 40, 50, and 60 ppm were prepared from this stock solution of 100 ppm.

100 µg/L $Zn^{2+}_{(aq)}$

Twenty-five milliters (25 mL) of 1000 ppm $Fe^{2+}_{(aq)}$ was measured into a 250 mL volumetric flask, and diluted to the mark with deionized water. This gives a stock solution of 100 ppm, working standards of 10, 20, 30, 40, 50 and 60 ppm were prepared from this stock solution of 100 ppm.

100 µg/L $Al^{3+}_{(aq)}$

Twenty-five milliters (25 mL) of 1000 ppm $Al^{3+}_{(aq)}$ was measured into a 250 ml volumetric flask, and topped to the mark with deionized water. This gives a stock solution of 100 ppm, working standards of 10, 20, 30, 40, 50, and 60 ppm were prepared from this stock solution of 100 ppm.

Minimum Recovery Diluent

Analar grade $NaCl_{(s)}$, 0.850 g was weighed into 100 mL volumetric flask. It was then dissolved by distilled water and then topped to the mark with deionized water. This constitutes 0.85% physiological saline

70% Ethanol

Analar grade Ethanol, 70 mL was measured into 100 mL volumetric flask and then diluted to the mark with deionized water.

Ammonium persulphate solution, 1 M

Ammonium persulphate, 114.100 g was dissolved in 500 mL volumetric flask and topped to the mark with deionized water. It was kept in the refrigerator.

2.5 M H_2SO_4

Concentrated H_2SO_4 (140 mL) was added to 700 mL deionized water, wen cool it was transferred into a 1L volumetric flask and diluted to the mark with deionized water..

1.75 M H_2SO_4

Concentrated sulphuric acid (97.0 mL) was slowly added to 700 mL distilled water, when cool it was transferred into a 1L volumetric flask and diluted to the mark with deionized water.

0.875 M $NaOH_{(s)}$

Sodium Hydroxide, $NaOH_{(s)}$, 17.500 g was weighed into a beaker and dissolved with 300 mL of distilled water, it was transferred into a 500 mL volumetric flask when cool, and topped to the mark with deionized water.

$AS_2O_{3\,(aq)}$, 0.025 M

Arsenious trioxide $AS_2O_{3(s)}$, 5.000 g and 25.000 g NaCl were weighed into 200 mL of 0.875 M NaOH solution. Slowly, 32 mL of concentrated sulphuric acid was added to the solution in an ice bath while stirring. When cool, 25.000 g of NaCl was added and then adjusted to 1 L with cold deionized water, this was stored with intermittent stirring to aid dissolution. The solution was stored in the dark.

Ceric ammonium sulphate (CAS) solution, 0.038 M

Ceric ammonium sulphate, (24.000 g) was weighed into 1L of 1.75 M H_2SO_4 solution. It was stored in the dark.

Standard iodine solution, KIO_3

The stock solution was prepared by dissolving 0.840 g KIO_3 in deionized water in 500 mL volumetric flask, and then topped to the mark with deionized water.

Using the dilution formular, 5 mL of the stock solution (solution A) was measured into a 500 mL volumetric flask and diluted to the mark to form solution (B). Both solutions were stored separately in brown plastic bottles in a refrigerator. Working standards were prepared by adding aliquots of 200, 400, 800, 1200, 2000 and 3000 μL of standard B separately into 100 mL volumetric flasks and diluted to the mark. The blank standard was also prepared. The standards are equivalent to iodine concentrations of 20, 40, 80, 120, 200 and 300 $\mu g/L$. They were stored in plastic bottles and refrigerated

30% KI $_{(aq)}$

Potassium iodide KI (30.042 g) salt was weighed into a beaker and dissolved with 100 mL of deionized water. It was kept in an amber bottle.

0.60% HCl $_{(aq)}$

Concentrated HCl, (3.00 mL) was measured into a 500 mL volumetric flask and the topped to the mark with deionized water.

0.1M $Na_2S_2O_3$ $_{(aq)}$

Sodium thiosulphate (3.950 g) $Na_2S_2O_3$ salt was weighed into a 250 mL volumetric flask, dissolved with 50 mL deionized water and topped to the mark. It was shaken to mix properly, and then kept. A concentration of 0.001M was prepared from the stock (0.1M).

Classical Titration Method for the Determination of Urinary Iodine

Each urine sample, (1 mL) was diluted with 20 mL of deionised water and the pH adjusted to 2.8 by the addition of 0.6% HCl dropwise then, 2 mL of 30% KI $_{(aq)}$ was added to convert all the iodate to elemental iodine. The liberated iodine was titrated with freshly 5 mL prepared sodium thiosuphate solution using 1% starch solution as the indicator.

The titre values obtained were used to calculate the concentration of iodine (I_2) in the urine samples in mg/L using the formula below:

$$I = (\text{titre value} \times 105.8 \times 1000 \times \text{df})/(\text{volume of water} \times 〚10〛^{\wedge}6) \quad (4)$$

Where

df = dilution factor

The value obtained was converted to $\mu g/L$. The results are found in Appendix B.

Sandell-Kolthoff Method for Urinary Iodine Determination (Method A)

Procedure

The refridgerated urine samples were allowed to attain room (laboratory) temperature of 25 ^{o}C. Each sample was stirred with glass rod to suspend the sediment. 25 mL of each sample was pipetted into a separate test tube. 1 mL of ammonium persulphate solution was added to each test tube, and the test tube heated for 60 min at 93^{o}C, after which they were cooled to room temperature. Then, 2.5 mL of arseniuos acid solution was added to each test tube and mixed thoroughly, and allowed to stand for 15 min to become colourless. 300 μL of ceric ammonium sulphate (CAS) solution was added to the tube at 25 s intervals with thorough mixing. The samples allowed to attain the room temperature to develop a yellowish colour. 1 mL of the mixture was taken and diluted to 25 mL in a 25 mL volumetric flask. Exactly 30 min after the addition of CAS solution to the first tube, its absorbance was read at 405 nm using UV-VIS spectrophotometry. The successive tubes were read similarly as when adding to CAS solution. The results are found in Appendices C – F.

The equipment was warmed for 45 mins and calibrated at the selected wavelength using sample blank. The sample was then placed in a quartz cuvette and the beam of allowed through it. The sample then absorbs the UV radiation depending on the concentration of the analyte. The intensity of the radiation therefore corresponds to the amount of the analyte in solution.

The Novel Method for Urinary Iodine Determination

Optimizing and Verifying the Experimental Procedure

20 mL of the urine sample was measured itno a 250 mL glass stoppered conical flask. Concentrated H_2SO_4 (factor A1) 1 mL and concentrated HCl (Factor B1) 3 mL were added seperately. The glass was stoppered, the mixture was digested for 30 min on a hot plate at a temperature of 100°C. After the digestion, the mixture was allowed to cool. 3 mL of 10% $KI_{(aq)}$ was then added to develop a yellowish to brownish colouration, after which 1 mL of 1% starch indicator was added, a bluish coloration then developed. The mixture was then titrated against 0.001M $Na_2S_2O_{3\ (aq)}$ solution to determine the concentration of iodine. The determinations were done in triplicate. Maintaining the combinations of 1: 3 for H_2SO_4: HCl, and 3 mL of KI $_{(aq)}$ and 1% starch indicator. The experiment was repeated seperately with 20% KI $_{(aq)}$ at 1, 2 and 3 mL starch indicator solutions, and each time the concentration of iodine found.

The experiment was repeated with acid combination of 1:2 and 1:1 respectively for H_2SO_4: HCl for activity A. The results are found in Table 60. For Activity B the experiment is done with acid combinaitons of 3:1, 2:1 and 1:1 at 2 mL each of KI $_{(aq)}$ at 10, 20 and 30% concentrations with 1% starch indicators at 1, 2 and 3 mL each. The results are found in Table 61.

Percent Recovery Determination of Novel Method

Prior to analyzing the urine samples, fifty urine samples (50) were digested separately and the iodine content in each was determined by the proposed method. Then 1.0 ppb of iodine was spiked to each sample and the percentage recovery of iodine determined. The urine, 30 mL was split into two portions of 15 mL each. To one portion 5 mL of water was added so that volume of water (V_{water}) equals volume of standard solution ($V_{standard}$) and was measured to get the concentration of iodine in the unspiked sample, ie, $C_{unspiked}$. To the other portion, 5 mL of 10000 ppm KIO_3 solution containing 50 mg/L of iodine was added. It was then allowed to equilibrate for 3 days in the dark. This was labeled sample A, after which the iodine concentration was determined as C_{spiked}. This procedure was repeated for samples B, C, D and E containing 40, 30, 20 and 10 ppm iodine respectively. Since the spike is diluted by the sample, the dilution equation was used to get the concentration added, ie, C_{added}. The percent recovery was calculated for each of the three analytical methods as:

$$\% \text{ Recovery} = (C \text{ spiked} - C \text{ unspiked})/(C \text{ added}) \times 100 \quad (5)$$

Determination of Real Samples using Optimal Conditions

Upon determination of the optimum experimental conditions, the urine samples were analysed as follows:

Acid digestion of the urine

Urine sample (1 mL) was diluted to 20 mL with distilled water. 1 mL concentrated H_2SO_4 was added followed by 1 mL concentrated HCl. The mixture was boiled (digested) in a glass-stoppered conical flask for 30 mins at

a temperature of 100°C on a hotplate. The equation of the reaction is presented below (I-P represents organic iodine; P represents organic residue)

Equation: $I\text{-}P_{(aq)} + H^{+}_{(aq)} \longrightarrow I^{-}_{(aq)} + P_{(aq)}$ (6)

Oxidation of iodide ion, I^{-}

Potassium permanganate, $KMnO_{4(aq)}$ 25 mL of 0.1M was placed into the flask dropwise whilst maintaining the temperature at 50°C until the purple colour of the $KMnO_4$ persists, it was followed immediately by adding 10 mL of 0.1 M solution of $K_2C_2O_4$ whilst still maintaining the reaction temperature at 50°C. The colour of the solution then changed from purple to colourless. The chemical equations are presented below

$$5H_2C_2O_{4\,(aq)} + 2M_nO_{4\,(aq)} + 6H^{+}_{(aq)} + I^{-}_{(aq)} \rightarrow Mn^{2+}_{(aq)} + CO_{2(g)} + H_2O_{(l)} + I_{2(aq)} \ldots \quad (7)$$

Titration of the I_2

1 mL of the colourless solution was then diluted to 20 mL followed by the addition of 2 mL 20% $KI_{(aq)}$ and 1 mL of 1% starch indicator. The resultant mixture was then titrated against 0.001M $S_2O_3^{2-}{}_{(aq)}$ to a colourless endpoint. A triplicate titration was performed, and the urinary iodine concentrations (UICs) were calculated as shown in Appendices F – 11.

The reaction equation is as below

$$I_{2\,(aq)} + S_2O_4^{2-}{}_{(aq)} \longrightarrow I\text{-}_{(aq)} + S_4O_6^{2-}{}_{(aq)} \quad (8)$$

Determination of Salt Iodine Content

Procedure

Prior to taking the salt sample, the sample was thoroughly mixed in a beaker to ensure that the iodine was homogenously distributed in the salt. Having prepared the solution, 25 mL aliquot was placed in a conical flask. 2 mL $H_2SO_{4\,(aq)}$ and 5 mL $KI_{(aq)}$ were added to the salt solution, the solution

turned yellow in the presence of iodine. The reaction mixture was kept in the dark for 10 min. 5 mL of starch indicator was added, and titrated against the thiosulphate solution until the bluish colouration of the solution turned colourless. The titration was performed in triplicate and the average titre value noted from the chart in Appendix M. The salt iodine concentrations are found in Tables 94 and 95.

Determination of Magnesium content of salt

This method involves precipitation, digestion and titration of magnesium with standard EDTA solution.

Precipitation of the Mg^{2+} from salt solution

The salt sample (50.000 g) was weighed and dissolved in deionized water. The solution was filtered and made up to 500 mL in a volumetric flask. 100 mL of the solution was pipetted out and diluted to about 150 mL, and acidified with six drops of concentrated HCl. 10 mL of 10% NH_4Cl (aq) and two drops of phenolphthalein indicator were added. The solution was made slightly ammoniacal with concentrated NH_3. It was then boiled and 15 mL of 10% ammonium oxalate solution ($(NH_4)_2C_2O_4$) added dropwise with stirring. The precipitate formed was digested and allowed to settle overnight, and filtered through a Whatman No. 40 filter paper and washed with cold water.

20 mL of the filtrate was pipetted into a conical flask, 2 mL of NH_3-NH_4Cl buffer solution (pH 10) and 2 drops of Eriochrome Black T indicator was added and titrated with the standardized EDTA. The procedure was repeated for the rest of the samples.The results are found in Tables 99 and 102 in Appendix N.

Standardization of the EDTA with $CaCO_{3\,(aq)}$

EDTA solution was titrated against standard $CaCO_3$ solution (primary standard) and the average titre was used to calculate the molarity of the EDTA. The magnesium content of the salt samples was calculated. The results are found in Tables 99 and 102 for unpackaged and packaged salts respectively.

Determination of Salt Moisture Content

The amount of salt was found by difference. The dish with its content was placed in an oven at a temperature of 105^{o}C for 6 hr, after which it was kept in a dessicator to cool and weighed. The procedure was repeated until constant weight was obtained, the moisture content was then found by difference. Having determined the moisture content, it was exposed to the weather for a week after which the amount of moisture absorbed by the salt was again found by difference, and hence the percent moisture calculated. The procedure was repeated with same sample until the end of the study. The results are found in Table 72 in Appendix O.

Determination of levels of Selected Metals in salt

The trace metals that were determined included lead (Pb), iron (Fe), cadmium (Cd), zinc (Zn) and aluminium (Al).

Acid Digestion of Salt Samples

10 mL HNO_3 was added to 2 mL H_2O_2 in 500 mL beaker. 1.000 g of dry salt sample were placed in a fume hood for 2 dys for digestion. The mixture was digested at 80^{o}C till the transparent solution was achieved. After cooling, the digested samples were filtered using buchnner funnel and the filterate was diluted to 250 mL with distilled water. The final solution was

used for the analysis of the heavy metals (Rajhi, 2014). Method blank samples were also analysed.

Analysis of the Trace Metals by AAS

The sample is fed into a flame to fragment into very small droplets (atomized). The hollow cathode lamp (appendix P) which contains the metal of interest emits light at a characteristic wavelength (appendix P) specific to the element being analysed (appendix P). This light passes over the flame (burner) to the detector, which measures the intensity of the emitted light. The intensity of the emitted light is proportional to the concentration of the analyte according to Beer's law (Rajhi, 2014).

Iodine Stability Determination

The stability determination was performed in two parts. One part was carried out in the laboratory (oven) under selected temperatures 28, 30, 100 and 180ºC (the reason is to find the effect of storage and cooking temperatures on iodised salt). The other part was carried out by exposing the salt to the weather. The import is to determine the contributions of moisture toward iodine stability, Table 72.

Microbial Studies in Salt

For each salt sample, 30 g was weighed and transferred to 180 mL of Minimum Recovery Diluent reagent. Samples were serially diluted through to the 10^{-2} by pipetting 1 mL of the sample into 9 mL of Minimum Recovery Diluent contained in McCartney bottle and mixed well.

Innoculation and Incubation

Duplicate dilutions of 1 mL of the initial suspension (10^{-1} and 10^{-2} dilutions) of each sample were transferred to each dish, by means of a sterile

micropipette into 20 mL of the plate count agar at 47°C into each Petri dish. The innoculum with the medium was mixed by rotating the petri dishes and allowed to solidify on a cool horizontal surface. The solidified prepared plates were inverted and incubated in an oven at 37°C for 28 hr. All colonies were counted, and an average of duplicate samples recorded as Total Variable Count (TVC) counts (CFU/mL) for the sample (ISO, 2015).

Similarly, colony count method by pour plate technique (ISO 4832: 2006) of inoculation was used. Two duplicate dilutions of 10^{-2} mL of each sample were plated with Eosin Methylene Blue agar and incubated at 37°C for 48 hr to observe for Total Coliforms. All purple colonies were counted, and an average of duplicate samples recorded as Total Coliforms counts (CFU/mL) for the sample (ISO, 2006a).

For yeasts and moulds counts, colony count method by pour plate technique (ISO 6611: 2004 E) was also employed. Duplicate dilutions of 1 mL of the initial suspensions (10^{-1} dilution) of each sample were transferred to each dish, by means of a sterile micropipette. 20 mL of the Potato Dextrose agar supplemented with Amoxicillin at 47°C into each Petri dish. The inoculum with the medium was mixed by rotating the Petri dishes and allowing the mixture to solidify by leaving the Petri dishes standing on a cool horizontal surface. The solidified prepared plates were inverted and incubated at room temperature for 5-7 dys. All colonies were counted, and an average of duplicate samples recorded as Yeast and moulds counts (CFU/mL) for the sample (ISO, 2006b).

Enumeration of Viable Bacteria Count

Growths of viable bacteria colonies observed on the cultured plates above were inspected and counted manually. Mean colonies calculated using the formula below (Maturin & Peeler, 2001).

$$N = \frac{\Sigma C}{[(1xn_1) + (0.1xn_2)x(d)} \quad (9)$$

where:

N = Number of colonies per ml or g of product

∑C = Sum of all colonies on all plates counted

n1 = Number of plates in first dilution counted

n2 = Number of plates in second dilution counted

d = Dilution from which the first counts were obtained

Chapter Summary

The research approach adopted was a combination of descriptive, experimental and fixed design. Sampling procedures were respectively convenient sampling and random sampling for urine and salt. The analytical procedure that were used to determine content of iodine in the salt and the urine samples were titration and Sandell-Kolthoff methods standard methods.

A method (Novel) was also proposed to determine the concentration of iodine in the urine samples due to the limitations of the Sandell-Kolthoff method. The factors that affect the stability of iodine in salt, presence of heavy metals and microbes in the salt were also studied.

CHAPTER FOUR

RESULTS AND DISCUSSION

Introduction

The purpose of this study is to determine the the causes of prevalence of iodine deficiency in Central region of Ghana. This is relevant since iodine deficiency still persists in this part of the country after the implementation of the mandatory iodisation law in 1996. The findings shall indicate the iodine status of the study populations and also point out the adequacy of iodine in the iodised salts that are consumed to prevent this deficiency. The research methods included the collection of urine and iodised salt samples, and the administering of questionnaire to volunteers.

One thousand and forty-eight (1,048) human urine samples were collected volunteers under the directives of University of Cape Coast (UCC) hospital staff. The analysis of the iodine in the urine samples was done using Sandel-Kolthoff, Classical method, and a proposed (Novel) method. Also, 126 samples of iodised salt made up of 92 packaged and 34 unpackaged salts were collected nationwide.The salt iodine content was determined by titration. The responses of the questionnaire were analysed by SPSS version 22 and STATA to determine behavourial patterns that could contribute to iodine deficiency in the Central region of Ghana.

Classical Titration Method for the determination of urinary iodine concentration (UIC)

A total of 48 urine samples were obtained from with the consent of the volunteers and by the permission of the Ethical Committee of the Institutional Review Board of University of Cape Coast.

The Urinary Iodine Concentration of Staff

It was found out that 7 of the samples, namely, samples 1009, 1016, 1023, 1025, 1026, 1033 and 1040 were found to be non-detect, Appendix B. This could mean that their iodine concentrations were below the detection limit of the method, or the iodine was present in such a state that was not readily available for detection by the method. Three (3) samples, Table 28, that were found to contain adequate iodine were samples 1041, 1046 and 1048. Their concentrations were respectively 148.42, 148.40 and 183.38 ppm at 95% sig, Appendix B. Such individuals in the population have sufficient iodine nutrition and adequate iodine status, Table 7. This assertion is strongly supported by the questionnaire responses since 97% of the staff consumed iodised salt to prevent iodine deficiency as 54% of them had knowledge about iodine deficiency, Table 73.

Table 28: Range of Urinary Iodine Concentration (µg/L) and Number of Staff

Range (µg/L)	Number of Staff
0-19	14
20-49	26
50-99	5
100-199	3
200-299	0
≥300	0

Source: Laboratory Results, Benjamin Bartels, University of Cape Coast (2017).

Moreover, 14 urine samples, Table 28, were found to contain urinary iodine less than 20 ppm. This implies such members of staff have insufficient

iodine intake and are severely iodine deficient, Table 7. They could belong to the 3% that donot consume iodised salt and or the 46% that donot have any knowlwdge about iodine deficiency according to the questionnaire responses, Table 73.

Table 29 summarises the questionnaire responses for the staff population.

Table 29: Summary of Questionnare Responses of Staff

Predictors	Staff
Number of respondents, n	48
Female, %	67.0
Male, %	33.0
Age range, yrs	20-69
Lieracy rate, %	100.0
Consumption iodised salt, %	97.0
Knowledge about iodine deficiency, %	54.0
Iodine status test (not tested), %	100.0
Knowledge about the Law, %	0.1
Consumption of raw cabbage, %	95.0
Consumption of millet, %	85.0
Consumption of peas, %	51.0
Smoking, %	0.0
Drinking water, %	95.0
Consumption of soyabean, %	69.0

Source: Field Work, Benjamin Bartels, University of Cape Coast (2017).

The median concentration obtained for the overall population was 30.21 ppm at 95% sig. Such concentration is an indication of insufficient iodine intake and a moderate iodine deficient status by the population, Table 7. This could be attributed to the high consumption of goitrogens (raw cabbage, millet, peas, soyabean and water) which are known to cause iodine deficiency (Gbadegbo & Nwufoh, 2010; Environmental Health Perspective, 2010). This is proven by the questionnaire responses which showed appreciable level of consumption of goitrogens by staff: 95% of raw cabbage, 85% for millet, 51% for peas 69% for soyabean and 95% for water, Table 73.

The urinary iodine concentration of pupils

The median urinary iodine concentration (MUIC) for the overall population, (n = 195) was found to be 43.46 ppm at 95% sig. This result indicates that the pupils have insufficient iodine nutrition, and moderate iodine deficiency as stipulated by WHO as stipulated in Table 7. The minimum concentration found was 8.48 ppm for 7 samples as shown in Table 29, this shows an insufficient iodine nutrition and severe iodine deficiency status as shown in Table 7.

There are, however, 4 urine samples as depicted in Table 29, which are samples 54, 75, 149 and 193 with respective urinary iodine concentrations of 190.80, 100.73, 116.61 and 106.44 ppm, Appendix B. These results show adequacy in iodine nutrition according to Table 11. The Seven (7) samples, Table 30, are made up of samples 107, 110, 115, 116, 133, 181, and 191 with respective urinary iodine concentrations of 18.02, 9.54, 15.94, 8.48, 18.02, 11.66 and 15.94 ppm, Appendix B, had severe iodine deficiency, Table 7.

The range of iodine concentrations and number of samples are shown in Table 29.

Table 30: Range of Iodine Concentration (µg/L) and number of Pupils

Range (µg/L)	Number of pupils
0-19	7
20-49	123
50-99	61
100- 199	4
200 – 299	0
>300	0

Source: Laboratory Results, Benjamin Bartels, UCC, (2017)

The urinary iodine concentration of undergraduates

The UIC of the population (n = 800) is shown in Appendix B for samples 196-995. The median UIC of the population was found to be 48.31 ppm at 95% sig. The value depicts that the population has insufficient iodine intake and a moderate iodine deficiency status as stated in Table 8. The minimum urinary iodine concentration found was 11.44 ppm, this is an indication of severe iodine deficiency Table 7. The maximum urinary iodine concentration was 106.56 ppm which shows adequate iodine intake.

A total of 790 urine samples were found in the iodine deficient range of 20-99 ppm, while 10 samples were found to be severely iodine deficient as shown in Table 30.

Table 31: Range of Urinary Iodine Concentration (µg/L) and Number of Undergraduates

Range (µg/L)	Number of undergraduates
0-19	10
20-49	399
50-99	391
100-199	0
200-299	0
≥300	0

Source: Benjamin Bartels, Laboratory Results, 2017, UCC

The urinary iodine concentration of household

The UIC for the population (n = 5) are found in Appendix B for samples 996 to 1000. The minimum urinary iodine concentration obtained was 10.58 ppm, and the maximum was found to be 99.60 ppm, whilst the median was 31.74 ppm at 95% sig as shown in Table 31. The median concentration suggests inadequate nutrition intake by the overall population. This classifies the household as a moderately iodine deficient population, Table 7.

Table 32: Range of Iodine Concentration (µg/L) and Number of Household Members

Range	Members of Household
0-19	1
20-49	3
50-99	1
100 -199	0
200-299	0
≥300	0

Source: Laboratory Results, Benjamin Bartels, UCC (2017)

The method specifically classified the populations as being iodine deficient. The Sandell-Kolthoff method for determining urinary iodine.

Iodine Status of Staff

Urinary iodine concentrations were obtained for the seven samples that the standard classical iodometric method was non - detect. The concentrations obtained were respectively 54.31, 47.3, 40.55, 40.83, 40.46, 42.46, and 40.46 ppm for samples 9, 16, 23, 25, 26, 33 and 40 provided in Appendix C. These concentrations, however, classified samples 9 and 16 as mildly iodine deficient, and samples 23, 25, 26, 33 and 40 as being moderately iodine deficient as shown in Table 7.

The minimum and maximum urinary iodine concentrations obtained for the population (n = 48) were respectively 40.46 and 341.87 ppm. The median concentration found for the overall population was 77.69 ppm at 95% sig. The minimum concentration of 40.46 ppm suggests that there are individuals within the population who are moderately iodine deficient as stated in Table 7. On the other hand, the maximum concentration of 341.87 ppm shows that among the population are also individuals who have excess iodine intake and are at risk of iodine toxicity, Table 7. Apart from these extreme values, the median value of 77.69 ppm indicates that the overall population has insufficient iodine nutrition and mild iodine deficiency status as shown in Table 7.

This is an improvement over the 30.21 μg/L (moderate iodine deficiency) obtained for the same population by the classical titration method, Appendix B. There were 10 samples which have adequate iodine intake and

are therefore iodine sufficient, Table 32. Alternatively, the standard iodometric method recorded no iodine adequacy for the same samples.

Table 33: Range of Urinary Iodine Concentration (μg/L) and Number of staff

Range	Number of staff
0-19	0
20 -49	13
50-99	23
100- 199	10
200 -299	0
>300	2

Source: Laboratory Results, Benjamin Bartels, UCC (2017)

Iodine status of pupils

The urinary iodine concentrations of pupils (n = 195) are found in Appendix D. The median urinary iodine concentration (MUIC) was found to be 102.08 ppm, Appendix C. This value suggests that the population has adequate iodine intake and an optimal iodine nutrition status as shown in Table 7. This concentration is contrary to 43.46 ppm urinary iodine concentration obtained by the classical titration method, which conferred insufficient iodine intake and moderately iodine status on the same population. The maximum and the minimum concentrations were found to be respectively 50.02 and 306.22 ppm respectively at 95% sig. The Sandell-Kolthoff recorded 98 samples, Table 33, that had adequate iodine nutrition and sufficient iodine status as shown in Table 11. This situation is contrary to the 4 samples obtained by standard iodometric method, Table 38.

Table 34: Range of Urinary Iodine Concentration (μg/L) and Number of staff

Range	Number of pupils
0-19	0
20-49	0
50-99	91
100- 199	98
200 – 299	4
≥300	1

Source: Laboratory Results, Benjamin Bartels, UCC (2017)

One sample had excess iodine intake, Table 33, this excess iodine intake could contribute to hypothyroidism in that individual because it could raise tyrosine stimulating hormone concentrations (Zimmermann, 2008) and might lead to childhood goitre (Zhao et al, 2000). The median value for the population was 102.08 ppm, which showed that the pupils had no iodine deficiency. Urban pupils such as studied in this work, could be under strict parental control with respect to diet. A similar study in Zimbabwe, however, showed that Zimbabwean pupils had concentrations ranging from 290 and 560 ppm, these concentrations are higher than what was found in this study which was 50 to 300 ppm. This observation implies that pupils in Zimbabwe are consuming excess iodine than their counterparts in Ghana and that the Zimbabwean pupils could suffer thyrotoxicosis.

Iodine Status of Undergraduates

Appendix E shows the UIC of the population (n = 800). The median UIC obtained was 106.74 ppm at 95% sig. This concentration is an indication of iodine sufficient population as shown in Table 7; whereas the classical

titration method however conferred moderately iodine deficient status on the same population. Moreover, the minimum urinary iodine of 33.72 ppm found puts such individuals in the moderately iodine deficient status, instead of the severely iodine deficient status (11.4 ppm) of the standard iodine method for the same population. About 58% (467 pupils) of the population was found to be within the iodine sufficient range, Table 34.

Table 35: Range of Urinary Iodine Concentration (μg/L) and Number of Undergraduates

Range	Number of Undergraduates
0-19	0
20-49	0
50-99	325
100-199	467
200-299	6
≥300	0

Source: Laboratory Results, Benjamin Bartels, UCC (2017)

Iodine Status of Household

Appendix F shows the urinary iodine concentrations (UIC) of the population (n, 5). The minimum urinary iodine concentration was found to be 70.28 ppm, such individuals have insufficient iodine intake. On the other hand, the maximum urinary iodine intake which was 93.78 ppm, suggested sufficient iodine intake according to Table 7. The median concentration for the overall population was 70.28 ppm at 95% sig, this concentration is a mild iodine deficiency status on the population. Alternatively, the classical titration

method puts the population median at 31.74 ppm, which is moderate iodine deficient status as shown in Table 7.

The Novel (Proposed) Method

Optimizing the Experimental Procedure

In the presence of starch, aqueous solution of iodine forms a bluish or purple colour that is a complex between starch (amylose) and the iodine. The intensity of the colour depends on the concentration of iodine, and the amount of starch and quantity of iodide that are added. Excess of $KI_{(aq)}$ is always required to solubilize all the free iodine present, but a large excess might affect the solutions response likewise the starch. Developing a standard method for determining iodine based on these considerations would require that their respective concentrations be optimised to give a maximum response. The concentrations and volumes of the digesting acids were also optimized, Tables 35 and 36.

To find the optimum experimental conditions response, initial set of factor levels were selected and their responses measured. These are various combinations of the digesting acids, H_2SO_4 (Coded A) and HCl (Coded B) and $KI_{(aq)}$ and the starch indicator. For instance, in Activity A with acid combination of 1:3, triplicate titrations were done, and the concentrations of iodine (response) found. Then the ratios of the digestion acids were varied as 1:2 and 1:1, and the respective concentration found, Table 35.

The response at 20% $KI_{(aq)}$ at an acid combination of A5 1mL, B5 1mL and 2mL 1% starch indicator gave a relatively higher response of 70.52 ppm iodine, Table 35.

Table 36: Activity an Initial Set of Average Factor Response at Different

Acid (mL)	Acid (mL)	$KI_{(aq)}$, 3 mL	$KI_{(aq)}$, 3mL	$KI_{(aq)}$), 3mL	Starch, 1%	Starch, 1%	Starch, 1%
H_2SO_4	HCl	10%	20%	30%	1 mL	2 mL	3 mL
A1 1	B1 3	58.10±0.02	57.43±0.00	56.93±0.03	57.43±0.01	57.43±0.03	56.93±0.00
A3 1	B3 2	57.80±0.01	58.70±0.04	58.96±0.01	58.70±0.04	59.21±0.03	58.70±0.06
A5 1	B5 1	61.38±0.01	70.52±0.02	70.49±0.05	70.52±0.03	70.52±0.02	70.52±0.04

Source: Laboratory Results, Benjamin Bartels, UCC (2017)

For Activity B, the ratios of the digestion acids were reversed and their responses with respect to iodine concentration were found, Table 36.

In Activity B, combination A6 1 mL B6 1mL also gave a maximum response of 70.90 ppm at 2 mL of 20% $KI_{(aq)}$ and 1 mL of 1% starch indicator in Table 36.

Table 37: Reversal of the Initial Set of Factor Response at Different Combinations of H2SO4 and HCl (Activity B)

Acid (mL)	Acid (mL)	$KI_{(aq)}$,2(mL)	$KI_{(aq)}$,2 mL	$KI_{(aq)}$, 2mL	Starch,(1%)	Starch,(1%)	Starch,(1%)
H_2SO_4	HCl	10%	20%	30%	1 ml	2 mls	3 mls
A2 3	B2 1	48.79±0.01	48.28±0.04	46.80±0.00	48.80±0.04	48.28±0.00	49.05±0.04
A4 2	B4 1	69.13±0.00	44.22±0.01	43.71±0.01	44.22±0.02	44.22±0.03	43.71±0.02
A6 1	B6 1	69.13±0.03	70.90±0.02	66.84±0.03	70.90±0.01	69.12±0.05	66.84±0.00

Source: Laboratory Results, Benjamin Bartels, UCC (2017)

As the level of factors A and B was changed from point 5 to point 6 at 20% $KI_{(aq)}$, there was a significant effect on the response according to Table 37.

Table 38: Two Dependent Factors

Factor A (mL)	Factor B (mL)	Response, µg/L	Response, µg/L	Response, µg/L
H_2SO_4	HCl	10%	20%	30%
A1 1	B1 3	58.10±0.02	57.43±0.00	56.93±0.03
A2 3	B2 2	48.79±0.01	48.28±0.04	46.80±0.00
A3 1	B3 2	57.80±0.01	58.70±0.04	58.96±0.01
A4 2	B4 1	69.13±0.00	44.22±0.01	43.71±0.01
A5 1	B5 1	61.38±0.01	70.52±0.02	70.49±0.05
A6 1	B6 1	69.13±0.03	70.90±0.02	66.84±0.03

Source: Laboratory Results, Benjamin Bartels, UCC (2017)

The observed effect in the response was 70.90 – 70.52 = 0.48. Using the first order empirical model the 2^2 factorial design was generated as shown in Table 39.

Table 39: Factorial Design

Run	A	B	A*	B*	Actual response
1	1	3	-1	+1	57.43
2	3	1	+1	+1	48.28
3	1	2	-1	+1	58.70
4	2	1	+1	-1	44.22
5	1	1	-1	-1	70.52
6	1	1	-1	-1	70.90

Source: Laboratory Results, Benjamin Bartels, UCC (2017)

Run: factor level | **A, B**: acid combinations | **A*, B*** : responses

This empirical response model was used in obtaining the calculated responses, Table 40.

Table 40: Actual Response and Calculated Response

Actual response	Calculated response	Difference of responses	Percent random error
57.43	59.30	1.87	3.2
48.28	55.12	6.84	14.2
58.70	54.50	4.2	7.1
44.22	52.40	8.18	18.5
70.52	71.34	0.82	1.1
70.90	71.34	0.44	0.62

Source: Laboratory Results, Benjamin Bartels, UCC (2017)

The actual responses of 70.52 and 70.90 ppm agreed with the calculated responses of 71.34 and 71.34 ppm respectively as shown in Table 39. This suggests that the interaction between 1 ml H_2SO_4 and 1 ml HCL gave an optimum response of 70.90 ± 0.62 ppm at 2 mL of 20% KI and at 1 mL 1% starch indicator as indicated in Tables 36 and 39.

Method Verification: Ruggedness Testing

The percentage analyte for A1:B1 found were found were R4 58.10; R5 57.43 and R6 56.93. The percentage analyte for A6 and B6 were R1 69.13; R2 70.90 and R3 66.84 as shown in Table 37. Using the relation E_A = ((R1+ R2+R3) /3)) – ((R4 +R5+R6) /3)), the effect of a change for each factor was calculated as provided in Table 41.

Table 41: Actual Response and Calculated Response

Factor	Effect of Change
Digesting time (A)	–11.24
Volumes of acids used (B)	– 3.66
Digesting temperature (C)	– 3.39
Concentrations of acid (D)	– 4.86
Volume of KI (aq) used (E)	– 5. 20
Source of heat (F)	+ 3.36

Source: Laboratory Results, Benjamin Bartels, UCC, (2017)

Ordering the factors by their absolute values the following order was obtained: A 11.24 > E 5.20 > B 3.66 > C 3.39 > F 3.36 > D 4.86. The values showed that to obtain higher results depends critically on the digesting time (A), and least on the source of heat as provided in Table 40.

Validating the Method

The validation of the method was done by determining the percentage recovery of the method, Table 42. The percentage of iodine recovered ranged from 101-125%, this is a good recovery by the method.

Table 42: Percent Recovery of Urinary Iodine by Standard Addition Method in Digested Urine Samples

Urine Sample	Amount of $I_{2(aq)}$ in $1mL$ digested urine sample ($10^{-3}\mu g\ I_2$)	Amount of $I_{2(aq)}$ in $1mL$ of spiked sample ($10^{-4}\ \mu g\ I_2$)	Total amount of $I_{2\ (aq)}$ ($10^{-4}\mu g\ I_2$)	Total $I_{2\ (aq)}$ Recovered ($10^{-4}\mu g\ I_2$)	Percentage Recovery (%)
1	106	1.00	1.061	1.065	106.6
2	285	1.00	2.851	2.852	100.2
3	272	1.00	2.721	2.739	102.5
4	152	1.00	1.521	1.5.30	101.7
5	193	1.00	1.931	1.940	101.1
6	304	1.00	3.041	3.050	152.0
7	381	1.00	3.811	3.824	101.7
8	266	1.00	2.661	2.680	102.9
9	75	1.00	0.751	0.759	101.8
10	69	1.00	0.691	0.698	101.7
11	124	1.00	1.241	1.260	103.7
12	24	1.00	0.241	0.248	102.9
13	20	1.00	0.201	0.206	102.5
14	54	1.00	0.541	0.548	101.3
15	57	1.00	0.571	0.583	102.1
16	34	1.00	0.341	0.373	109.4
17	275	1.00	2.751	2.763	101.6
18	247	1.00	2.471	2.488	103.6
19	268	1.00	2.681	2.692	101.6
20	136	1.00	1.361	1.382	105.8
21	177	1.00	1.771	1.782	101.4
22	92	1.00	0.921	0.942	102.3
23	83	1.00	0.831	0.803	106.3
24	130	1.00	1.301	1.386	128.2
25	52	1.00	0.521	0.540	103.6
26	42	1.00	0.421	0.436	103.6

Table 41: Continued

27	54	1.00	0.541	0.569	105.2
28	120	1.00	1.121	1.140	115.7
29	186	1.00	1.861	1.889	103.3
30	174	1.00	1.741	1.780	105.2
31	186	1.00	1.861	1.886	102.9
32	88	1.00	0.881	0.883	100.2
33	32	1.00	0.321	0.340	105.9
34	178	1.00	1.781	1.784	100.3
35	128	1.00	1.281	1.288	102.4
36	330	1.00	3.301	3.308	102.3
37	269	1.00	2.691	2.698	101.3
38	118	1.00	1.181	1.443	118.1
39	268	1.00	2.681	2.684	100.4
40	93	1.00	0.931	0.986	105.9
41	167	1.00	1.671	1.683	101.8
42	252	1.00	2.521	2.540	103.6
43	166	1.00	1.661	1.675	102.1
44	154	1.00	1.541	1.562	103.9
45	143	1.00	1.431	1.499	115.8
46	73	1.00	0.731	0.750	102.6
47	184	1.00	1.841	1.883	104.9
48	196	1.00	1.961	1.985	100.4
Control urine	0	1.00	0.981	0.984	100.3

Source: Laboratory Results, Benjamin Bartels, UCC, (2017)

Proposed Epidemiological Criteria for Determining Iodine Status

The criteria, Table 43, were developed to determine the iodine status of the study populations. This was important because the urinary iodine concentrations obtained by the method were much higher than those provided by WHO in Table 5.

Table 43: Novel Epidemiological Criteria for the Determination of Iodine Status

Median urinary iodine concentration, µg/L	Iodine nutrition
<150	Poorly Deficient
150-399	Fairly Dificient
400-649	Firely Sufficient
650-869	Ideal
870-1100	Optimally Sufficient
≥1100	Excess

Source: Laboratory Results, Benjamin Bartels, UCC, (2017)

Analysis of the Urine Samples by the Novel Method

Having established the optimum experiemtnal conditions and the epidemiological criteria, the urine samples were determined using the novel method.

Iodine Status of Staff

The UIC of the staff population (n = 48) are found in Appendix H. Seven (7) of the urine samples namely sample 9, 16, 23, 25, 26, 33 and 40, Appendix B, were found to contain respectively 552.96, 534.53, 421.89, 354.30, 235.52, 204.80 and 421.89 ppm, Appendix H. These concentrations are about 10 times higher than those provided by the Sandell-Kolthoff method for the same samples, which are respectively 54.31, 47.31, 40.55, 40.83, 40.46, 40.46 and 40.46 ppm, Appendix C. The novel criteria, Table 42, classified the concentrations as ranging from fairly deficient to fairly sufficient iodine status. The maximum and minimum concentrations were respectively 3379.20 and 102.40 ppm.

The median concentration obtained for this population is 616.96 ppm. This classified the population as optimal in iodine nutrition as shown in Table 43. This is contrary to the median concentration of 77.69 ppm (mildly iodine deficient, Table 7), by Sandell-Kolthoff method; and 30.21 ppm (moderate iodine deficiency, Table 7). The maximum urinary iodine concentration was 3379.20 ppm, an indication of excess iodine nutrition, Table 42. This may be due to the extreme ignorance (100%) of their iodine consumption as shown in Table 7. The standard iodometric method provided a maximum of concentration of 183.38 ppm (adequate iodine status, Table 7) for the same samples.

Iodine Status of Pupils

Appendix 8 shows the results of UIC for the population (n = 195). The minimum urinary iodine concentration was 123.92 ppm. Such individuals have sufficient iodine intake and optimal iodine status, Table 44. The maximum UIC was 1219.20 ppm. This intake is in excess, Table 44, and could cause iodine toxicity according to. The median urinary iodine concentration was found to be 416.56 ppm, which classified the population as optimal. On the contrary, the Sandell-Kolthoff gave a median value of 102.08 ppm, and classified the the same population as having adequate iodine intake, Table 7. The titration method reported a concentration of 43.46 ppm, which is an indication of moderate iodine deficiency, Table 7.

Table 44: Epidemiological Range for Determining Iodine Status of Pupils

Range, µg/L	Iodine Nutrition
<90	Severe
90-900	optimal
>900	Excess

Source: Laboratory Results, Benjamin Bartels, UCC, (2 017)

Zhao et al, (2000), Delonge, (2000), and Guttinkonda, (2000) observed that such a population with sufficient iodine intake may not develop childhood goitre, speech and hearing impairment and cretinism. Medani et al., (2011) intimated that such a population could have high IQ and improved school performance. Analysis of questionnaire responses is shown in Table 45.

Table 45: Pearson's Correlations

Predictors		Academic performance	Raw cabbage	Iodised salt
Sig. (1-tailed)	Academic perfomance	1.000	-0.003	0.117
	Raw cabbage	**-0.003**	1.000	-0.019
	Salt iodised	**0.117**	-0.019	1.000
	Academic perfomance	1.000.	0.484	0.063
	Raw cabbage	0.484	1.000.	0.405
	Salt iodised	0.063	0.405	1.000.
n	Academic perfomance	172	172	172
	Raw cabbage	172	172	172
	Salt iodised	172	172	172

Source: Field Work, Benjamin Bartels, UCC, (2017)

There is a weak negative correlation between academic performance and cabbage consumption, r = -0.003, p = 0.484. This suggests that as the consumption of cabbage by the pupils (raw) reduces, their academic performance increases. This is because cabbage contains goitrogens called goitrins and oxazolidenes that prevent the uptake of iodine by the thyroid gland improves the IQ according to Guttnkonda, (2014) and Hess, (2010).

There was however a positive correlation though very weak, between academic performance and iodised salt consumption with r = 0.117 at p = 0.063, Table 44. This situation suggests that as consumption of iodised salt increases, academic performance increases as well, and vice versa. This observation has been highlighted by Fisher et al., (2011).

The Iodine Status of Undergraduates

The median concentration was found to be 622.30 ppm. The population is therefore fairly iodine sufficient as shown in Table 43. The standard iodometric method also estimated the population as having insufficient iodine intake and moderate iodine difficiency, Table 30. However, the Sandell-Kolthoff defines the population as being iodine sufficient according to Appendix E.

The minimum concentration of 213.36 ppm defines a poorly deficient iodine status, Table 45. The maximum concentration of 1351.28 ppm is an indication of optimum iodine intake and optimal iodine status according to Table 42. The behavioural pattern responsible for the iodine sufficiency is due to the high percentage (97%) that consumed iodised salt, and the moderate number (75%) that knows the improtnace of iodised salt in eliminating IDD, Table 45. However, ignorance about the salt iodisation law, lack of iodine

status test, and high consumption of goitrogens by the undergraduates could erode the gains, Table 46.

The questionnaire responses showed that 91% of the respondents consumed raw cabbage; and 70% consumed millet, 2% smoke, 79% consumed soy foods and 56% ate peas as provided in Table 46. These are goitrogens consumption of which brings about IDDs (Anderson et al., 2007).

Table 46: A Summary of Survey Responses of Undergraduate Students

Predictors (%)	Undergraduates
Number of respondents, n	179
Consumption of iodised salt	97
Knowledge about iodine deficiency	75
Iodine status test (not tested)	91
Knowledge about the law	21
Consumption of goitrogens (raw cabbage)	91
Consumption of goitrogens (millet)	70
Consumption of goitrogens (peas)	56
Consumption of goitrogens (smoking)	2
Consumption of goitrogens(drinking water)	99
Consumption of goitrogens(soyabean)	79

Source: Field work, Benjamin Bartels, UCC, (2017)

The Iodine Status of Household

Appendix L shows the urinary iodine concentration (UIC) of the population. The minimum urinary iodine concentration was 152.40 ppm, and the maximum concentration was 406.40 ppm. The median concentration was

264.16 ppm, the median value suggests that the population is fairly iodine deficient. The classical titration method also gave a median of 31.74 ppm.

Comparative Studies of the Analytical Methods

The results obtained from the various methods were statistically analysed to determine the most reliable results to predict the iodine status of the study populations. This was necessitated because the results obtained were different for the methods. The Novel method for instance gave consistently higher results because of the use of harsh digesting agents which release more organic bound iodine into solution.

This was carried out using the Horwitz Ratio, Pearson's Correlation and Discriminant analysis.

The Horwitz Ratio

The Horwitz ratio (HorRat) indicates the acceptability of methods of analysis. It is the ratio of the observed relative standard deviation (RSDR) calculated from the actual performance data to the corresponding predicted relative standard deviation, (PRSDR (%)), calculated from the Horwitz equation: 2c (- 0.15)) , where c is the concentration found expressed as a mass fraction). Deviations from the ratio on the low side (values < 0.5) and up to 1.0 may indicate satisfactory method of analysis whilst consistent deviations on the high side (values > 2) may indicate unsatisfactory analytical method (Horwitz&Albert, 2006,) [PubMed].

The summary of the results is shown in Table 46.

Table 47: Summary of the Results of the Horwitz Ratio

Samples	CTM		NOVEL		S -K	
	mean	s	mean	s	mean	s
Staff	34.34	36.64	576.80	483.95	96.41	74.95
Pupils	49.16	24.43	596.77	264.41	108.97	45.73
Undergraduate	51.71	18.50	653.55	207.13	110.18	30.12
Household	41.08	31.11	295.25	106.00	71.78	19.42
Total	176.78	110.74	2122.37	1061.49	387.34	169.72
Average	44.07	27.69	424.47	267.37	96.84	42.43
RSD_{actual}		62.8		49.16		43.8
$RSD_{predicted}$		44.07		424.47		96.8
$HorRat_{(ratio)}$		**1.40**		**0.12**		**0.45**

Source: Laboratory Results, Benjamin Bartels, UCC (2017)

RSD_{actual} = performance standard deviation

$RSD_{predicted}$ =standard deviation according to Horwitz equation

RSD = relative standard deviation = (100 x s) / average of means

s = standard deviation

The obtained ratios were 1.4, 0.12 and 0.5 respectively for classical titration method, novel method and Sandel-Kolthoff as shown in Table 46. Since values less than 0.5 and a maximum of 1.0 indicate satisfactory method of analysis, and values greater than 2 may indicates unsatisfactory method. (Horwitz & Albert, 2006) [PubMed]. It implies that in this study, the Novel method produced the most reliable results, followed by Sandell-Kolthoff method and then the standard iodometric method.

Pearson Correlation

The Pearson's correlation returned a positive correlation of 0.613 at 0.01 between the Novel method and the Classical titration method; and 0.612 at 0.01 between Sandell-Kolthoff and the Classical titration method. However, there was a much stronger positive correlation 0.946 at 0.01 between the Sandell-Kolthoff and the Novel methods. This is an indication that these two methods are comparable. The skewness of the classical titration method was 0.799, then 1.217 and 1.116 respectively for novel method and Sandell-Kolthoff respectively. These values were above zero, indicating that the populations do not have a normal distribution. The data therefore has a right skewed distribution with most values concentrated on the left, and most extreme values on the right as provided by Table 47 and Figures 12, 13 and 14.

Table 48: Statistics of the Analytical Methods

	Classical titration method	Novel method	Sandell – Kolthoff
Skewness	0.799	1.217	1.116
Kurtosis	2.966	4.8336	4.108

Source: Laboratory Results, Benjamin Bartels, UCC (2017)

The Novel method had kurtosis of 4.833. This is >3 and thus have leptokurtic distribution. The values then concentrate around the mean, with high probability for extreme values, Figure 12.

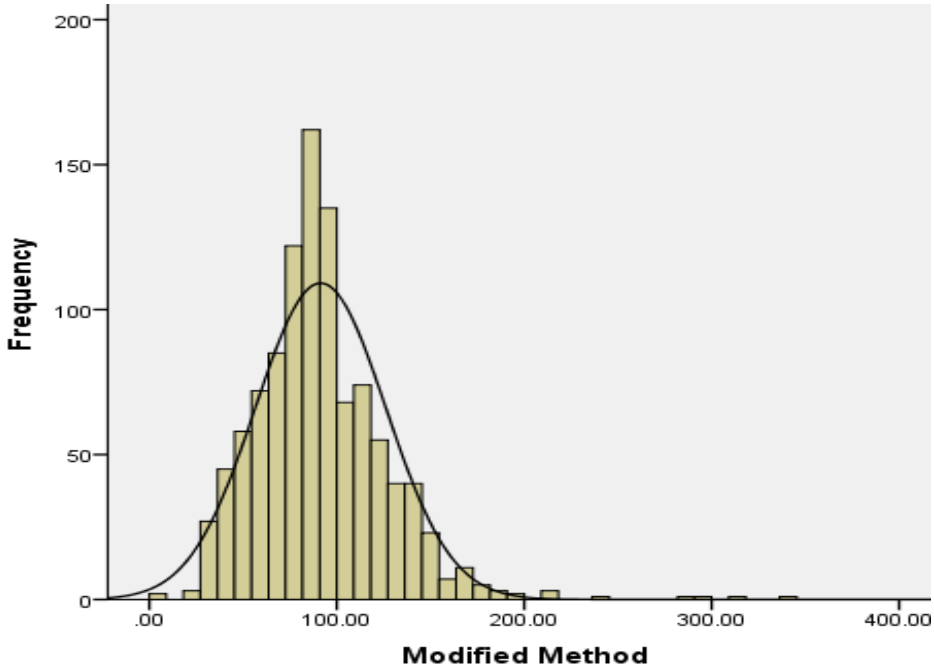


Figure 12: Leptokurtic Distribution of the Novel Method.

Sandell-Kolthoff yielded kurtosis of 4.108, also >3 and had leptokurtic distribution with high prbability for extreme values, Figure 13.

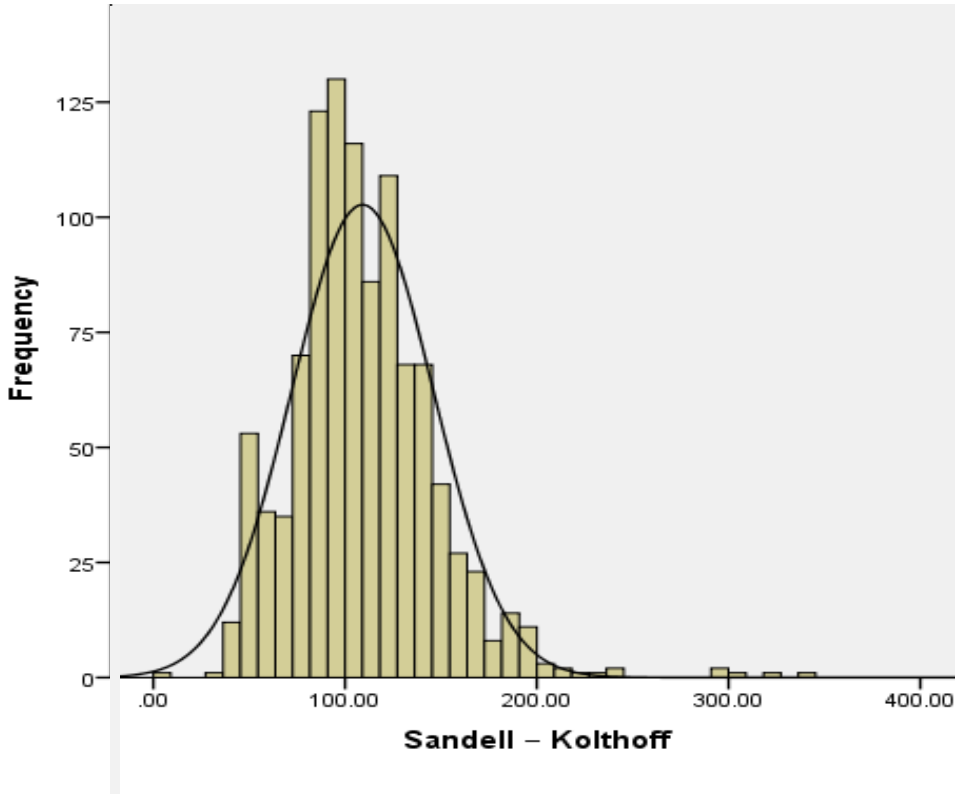


Figure 13: Leptokurtic Distribution of the Sandell-Kolthoff Method.

Kurtosis of 2.97 was obtained for the standard method. This value <3 and thus presents a platykurtic distribution of which the probability for extreme values is less than for a normal distribution, and the values are wider spread around the mean, Figure 14.

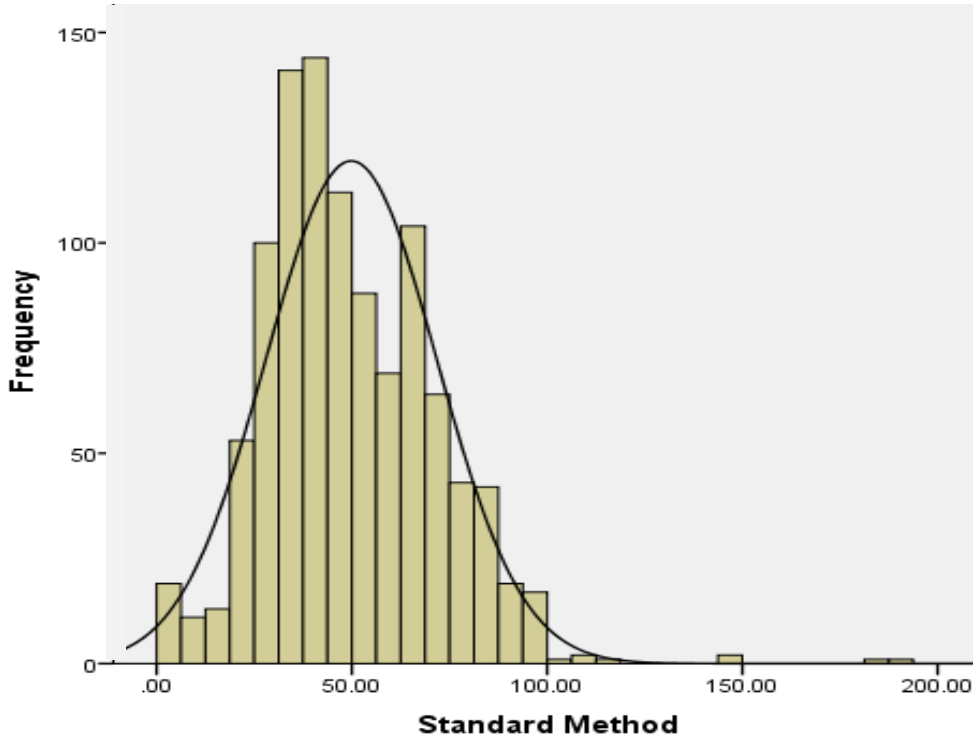


Figure 14: Platykurtic Distribution of the Classical titration method.

Discriminant Analysis

The analytical results (urinary iodine concentrations) were also subjected to Discriminant analysis to check if they have been correctly classified or assigned to the populations, Table 48.

Table 49: Group Statistics

Subgroup of Respondents		Mean	Std. Deviation	Valid N (listwise)	
				Unweighted	Weighted
1	Classical titration method	49.1558	24.49764	194	194.000
	Novel method	596.77	264.41	194	194.000
	Sandell – Kolthoff	108.9707	45.84444	194	194.000
2	Classical titration method	51.0526	19.47267	801	801.000
	Novel method	653.55	207.13	801	801.000
	Sandell-Kolthoff	110.0451	30.36779	801	801.000
3	Classical titration method	24.3360	12.72797	5	5.000
	Novel method	413.34	146.00	5	5.000
	Sandell – Kolthoff	61.7940	22.44422	5	5.000
4	Classical titration method	34.7379	37.33019	47	47.000
	Novel method	576.80	483.95	47	47.000
	Sandell – Kolthoff	97.8164	75.94443	47	47.000
Total	Classical titration method	49.8412	21.84521	1047	1047.000
	Novel method	638.32	239.17	1047	1047.000
	Sandell – Kolthoff	109.0667	36.95646	1047	1047.000

Source: Laboratory Results, Benjamin Bartels, UCC (2017)

1. refers to pupils 2. refers to undergraduates 3. refers to household
4. refers to staff

The results of the Novel method gave relatively higher mean points for all the paired populations with the exception of pupils/staff pair which was discriminated better by the Classical titration method, Table 50.

Table 50: Difference between Means of Populations

Populations	Difference	Between	Means
	Classical titration method	Novel method	Sandell-Kolthoff method
pupils/undergraduates	2.66	56.78	1.50
pupils/household	34.75	183.43	66.05
pupils/staff	20.19	19.97	15.62
undergraduates/household	37.39	240.21	67.55
undergraduates/staff	22.83	76.75	17.11
household/staff	14.56	163.46	50.44

Source: Laboratory Results, Benjamin Bartels, UCC, (2017)

'The test of equality score' is shown in Table 51.

Table 51: Tests of Equality of Group Means

	Wilks' Lambda	F	df1	df2	Sig.
Classical titration method	0.969	10.949	3	1043	0.000
Modified method	0.984	5.668	3	1043	0.001
Sandell – Kolthoff	0.987	4.409	3	1043	0.004

Source: Laboratory Results, Benjamin Bartels, UCC, 2017

A high Wilks's lambdas of 10.949 were obtained for classical titration method, and relatively lower F, 5.668 and 4.409 respectively for novel method, and Sandell-Kolthoff. The pooled variance within-groups matrices suggests use of these as intercorrelations are low 0.608 for Sandell-Kolthoff and classical titration method, and 0.618 novel method and classical titration

method as depicted in Table 52.

Table 52: Pooled Within-Groups Matrices

		Classical titration Method	Novel method	Sandell – Kolthoff Method
Correlation	Classical Titration method	1.000	0.618	0.608
	Novel method	0.618	1.000	0.952
	Sandell – Kolthoff method	0.608	0.952	1.000

Source: Laboratory Results, Benjamin Bartels, UCC (2017)

The Log determinants result is found in Table 53.

Table 53: Log Determinants

Subgroup of Respondents	Rank	Log Determinant
1. Pupils	3	17.826
2. Undergraduate	3	17.106
3. Household	3	11.401
4. Staff	3	18.986
Pooled within-groups	3	17.580

Source: Laboratory Results, Benjamin Bartels, UCC, (2017)

The pupil's subgroup had 17.8, with undergraduates having 17.1. The staff sub population had 18.9, and the household had a variant of 11.40; and Box M is 292.156 with F equals 14.241 significant at $P < 0.000$, Table 54.

Table 54: Test Results

Box's M		292.156
F	Approx.	14.241
	df1	18.0
	df2	800.667
	Sig.	0.000

Source: Laboratory Results, Benjamin Bartels, UCC (2017)

'Tests of Null hypothesis' of equal population covariance matrices. The Eigen values are found in Table 55.

Table 54: Eigenvalues

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	0.151[a]	84.6	84.6	0.363
2	0.024[a]	13.5	98.0	0.153
3	0.004[a]	2.0	100.0	0.059

Source: Laboratory Results, Benjamin Bartels, UCC, (2017)
a. First 3 canonical discriminant functions were used in the analysis.
At $p < 0.000$, H_0 is rejected and H_1 accepted.

Function 1 has the largest Eigen value of 0.151 and the highest canonical correlation of 0.363 even though it is comparatively low when compared with a standard of 1 as displayed in Table 83. Therefore testing the hypothesis regarding the discriminating power in the variables by Wilk's Lambda, Table 55, Ho is rejected.

Table 55: Wilks' Lambda

Test of Function(s)	Wilks' Lambda	Chi-square	df	Sig.
1 through 3	0.845	175.531	9	0.000
2 through 3	0.973	28.509	4	0.000
3	0.996	3.665	1	0.056

Source: Laboratory Results, Benjamin Bartels, UCC (2017)

H_0: there is no significant discriminating power in the variables

H_1: there may be a significant discriminating power in the variables

The relative importance of each independent variable was checked by comparing the standardized coefficient, Tables 56 and 57. Based on the coefficients, the relative important predictor variables are as follows Sandell-Kolthoff (1.314), novel method (0.799), and classical titration method (0.461) Table 56.

Table 56: Standardized Canonical Discriminant Function Coefficients

	Function		
	1	2	3
Classical titration method	0.461	0.936	-0.735
Novel method	-3.212	0.799	-0.061
Sandell – Kolthoff	2.905	-0.756	1.314

Table 57: Structure Matrix

	Function		
	1	2	3
Classical titration method	0.241	0.970*	0.026
Sandell – Kolthoff	0.127	0.573	0.809*
Novel method	-0.162	0.658	0.736*

Source: Laboratory Results, Benjamin Bartels, UCC, (2017)
*Largest absolute correlation between each variable, and any discriminant function.

It is revealed that 77.2% of the cross-validated respondents are correctly classified by the novel method into pupils, undergraduates, household and staff. Actually pupils had 91.8%, undergraduates had 98.6%, household had 100% whilst staff had 95.7% as shown in Table 58.

Table 58: Classification Results[a,c]

Count %	Subgroup of Respondents	Predicted Group Membership			
		1	2	3	4
Original	1	17.0	177.0	0.0	0.0
	2	5.0	791.0	0.0	5.0
	3	0.0	5.0	0.0	0.0
	4	0.0	45.0	0.0	2.0
	1	8.8	91.2	0.0	0.0
	2	0.6	98.8	0.0	0.6
	3	0.0	100.0	0.0	0.0
	4	0.0	95.7	0.0	4.3
Cross-validated[b]	1	16.0	178.0	0.0	0.0
	2	5.0	790.0	0.0	6.0
	3	0.0	5.0	0.0	0.0
	4	0.0	45.0	0.0	2.0
	1	8.2	91.8	0.0	0.0
	2	0.6	98.6	0.0	0.7
	3	0.0	100.0	0.0	0.0
	4	0.0	95.7	0.0	4.3

Source: Laboratory Results, Benjamin Bartels, UCC, (2017)

Also, 77.4% of the original validated cases were correctly classified with actual classification being 91.2%, 98.8%, 100% and 95.7% respectively, Table 59.

Table 59: Classification Results[a,c]

		Subgroup of Respondents	Total
Original	Count	1	194
		2	801
		3	5
		4	47
	77.4%	1	100.0
		2	100.0
		3	100.0
		4	100.0
Cross-validated[b]	Count	1	194
		2	801
		3	5
		4	47
	77.2%	1	100.0
		2	100.0
		3	100.0
		4	100.0

a. 77.4% of original grouped cases correctly classified.
b. Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.
c. 77.2 % of cross-validated grouped cases correctly classified by the method.

Since it has been established that 8 (7.7) out of 10 volunteers in each group (population), Tables 58 and 59, have been correctly assigned their urinary iodine concentrations by the Novel method, the prediction of the iodine status

of the study populations shall be based on the results from the Novel method. Therefore, apart from the household population which is deficient, the staff, pupils and undergraduates populations have adequate iodine nutrition.

Salt Iodine Content

The salt iodine content was determined for both packaged and unpackaged iodised salt. Ninety-four (94) samples of packaged salts were analysed for their iodine content. They were made up of five imported brands, namely Concord plus, Salnova, Cerebos, Buffalo and Ritebrand; and five local brands which were Mr. Chef, U2, Lele, Ante Dede and Annapurna. The salt samples were collected from selected market centers across Ghana. The reason being that the salt are either exposed to the weather or not properly stored at the market centers. This affects the iodine concentration in the salt.

Packaged salt

This type of salt is sold in PVC packaging materials as shown in Plate 4.

Plate 4: Samples of Packaged Salt.

Iodine Content

The iodine content of the salt is shown in Table 60. The iodine content of the packaged salt samples obtained from super market in the West Hill Mall

in the Greater Accra Region was found to be relatively higher than those of the same brands found in the open markets.

Table 60: Concentration of Iodine in Packaged Salt from Selected Markets in Ghana

Region	Market	Brand of salt	I_2, mg/kg
Greater Accra	Accra Mall	A Concord Plus	33
		B Salnova	40
		C Cerebos	35
		D Buffalo	10
		E Ritebrand	non-detect
		F Annapurna	52
	Agbogbloshie	G Mr Chef	54
		H U2	50
		F	51
		A	28
	Malata	F	50
		A	25
		H	53
	Kaneshie	F	50
		H	53
		C	33
		G	56
		I Lele	40
	Makola	H	52
		A	29
		F	51
	Madina	G	54
		F	50
		H	53
		A	28
	Nima	F	51
		H	52
	Ashiaman	F	50
		G	55
		A	28
		H	52
	Tema	F	50
		G	52
		I	45
	Sege	F	51
		H	54

Table 60: continued

	Kasei	F		51
		A		28
Volta	Ho	F		49
		H		45
		A		28
	Aflao	F		50
		H		46
	Blekusu	F		50
		H		45
	Adina	F		50
		H		46
	Anlo- Afianyegba	F		51
	Anloga	F		51
		G		56
	Dabara	H		48
		G		54
		F		51
Northern	Tamale	F		51
		H		48
Upper West	Wa	F		50
		H		52
Upper East	Bolgatanga	F		49
		G		55
Brong Ahafo	Sunyani	F		51
		A		28
		C		35
Eastern	Koforidua	F		51
		I		45
		G		52
	Krobo Odumase	G		27
		A		24
		J	Ante dede	47
		F		50
		H		50
	Akim Oda	F		50
		H		53
		C		31

Table 60: Continued

Ashanti	Kumasi	F	51
		G	52
		H	52
		A	29
Central	Cape Coast	F	50
		G	52
		H	55
		J	47
	Kasoa	F	50
		G	50
		H	50
		A	30
Western	Takoradi	F	50
		H	45
		A	30
	Axim	H	53
		A	32
		F	51
	Half Assini	F	51
		H	53
		A	32

Source: Laboratory Results, Benjamin Bartels, UCC, (2017)

For instance, iodine content in Concord plus was 33.0 ppm at the Mall, and ranging from 24 to 33 ppm in the open markets. Cerebos also had iodine concentration of 35 ppm at the mall, but the concentration in the samples from the open markets ranged from 31 to 35 ppm. Annapurna also had iodine concentration of 52 ppm at the mall, and between 49 to 52 ppm at the open markets. This variation in the concentration of the salt could be due to the storage conditions and the weather as found in this study, Tables 96 and 97. This observation collaborates the findings by Benoist et al., (2004).

The iodine concentrations of the salt samples in the open markets were generally found to vary among the same brands as shown in Table 60.

For instance, the concentration of sample H (U2) in Agbogbloshie market was 50.0 ppm, but in the Kaneshie, Sege, Makola and Ho the concentrations were respectively 53, 52, 54 and 45 ppm.

On the other hand, average concentrations calculated, Table 61, showed that most of the salt were within the legal limit set by GSA.

Table 61: Average Concentrations, mg/kg of Iodine in Salt

Brand of salt	Average concentration
Concord plus	29.0 ± 0.03
Salnova	40.0 ± 0.00
Cerebos	34.0 ± 0.01
Buffalo	10.0 ± 0.04
Ritebrand	Non - detected
Annapurna	50.0 ± 0.02
Mr. Chef	51.0 ± 0.04
U2	51.0 ± 0.02
Lele	43.0 ± 0.02
Ante Dede	47.0 ± 001

Source: Laboratory Results, Benjamin Bartels, UCC, (2017)

For example, the average concentration of Annapurna salt, 50.0 ± 0.02 is found to be within the limit of 25-50 ppm as shown in Figure 15. Alternatively, U2 had an average concentration of 51 ppm which is slightly above the range, whilst Lele and Ante Dede recorded concentrations below the range as depicted by Figure 15.

Figure 15 shows the average concentrations of salt and the legal limits of salt iodine concentrations set by GSA.

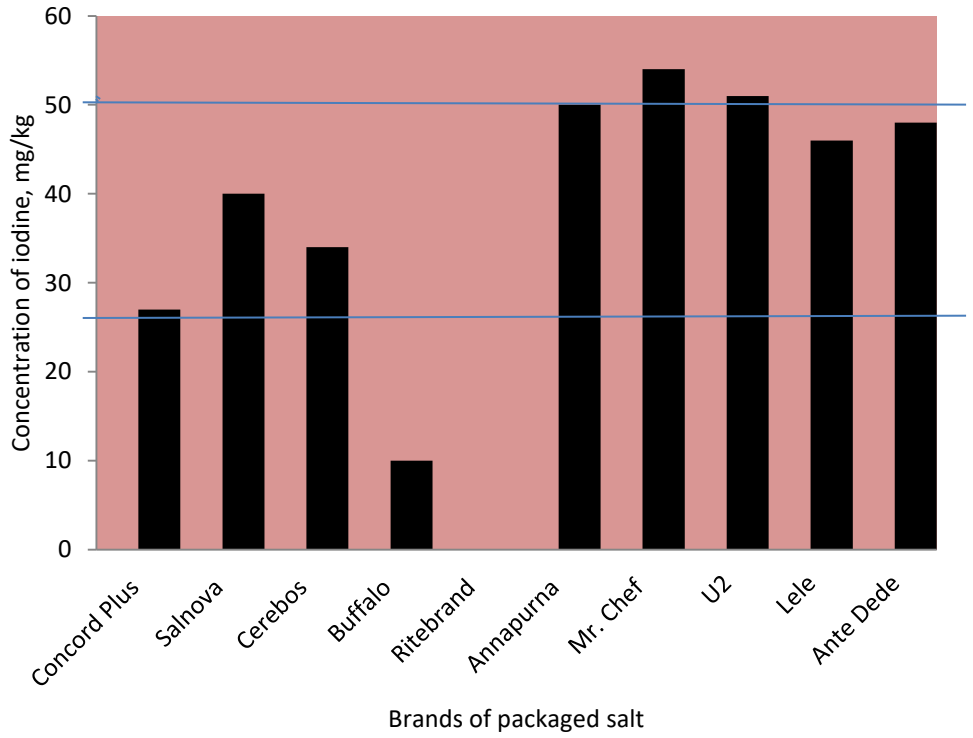


Figure 15: Average Concentrations and Legal Limits of Iodine in Iodised Salt.

As indicated in Figure 15, 60% of the salt analysed were found to contain adequate iodine at the retail level. Such samples included concord plus, Salnova, Cerebos, Annapurna, Lele and Ante Dede, their iodine concentrations were within the legal limit of 25-50 ppm as shown in Figure 15. These samples are deemed to have adequate iodine to fight IDD.

On the other hand, Mr. Chef and U2 had concentrations that were above the maximum legal limit of 50 ppm, Figure 15. This implies that they contained iodine in excess of what is required to fight IDD, and that its consumption could lead to iodine poisoning. These salts constitute 20% of salt analysed.

However, one percent (1%) of the salt analysed did not meet the requirement, this was Buffalo. Buffalo with iodine concentration of 10 ppm, which was below the legal limit of 25 ppm did not contain adequate amount of iodine to fight IDD. Ritebrand with non-detect is below the legal limit of 25 ppm, and did not contain adequate amount of iodine to fight IDD. Another 1% made up of Ritebrand could not have its iodine concentration detected by the method.

These findings collaborated studies by Buxton & Baguuna, (2012) who found that in the Western Region of Ghana, 58% of iodised salts contained less than 20 ppm iodine compared with the mandatory iodisation level of 25-50 ppm. The consumption of such salt could result in hypothyroidism and hyperthyroidism and malfunctioning of the body as observed by (Stephanie, 2014)

According to earlier findings, such variation in iodine concentration could arise from variability in the amount of iodine added. The rest are uneven distribution of iodine in the iodised salt within batches and individual bags, and packaging during production. Variation could also be attributed to salt impurities and environmental conditions during storage and distribution. Since 60% of the iodised salt investigated for their iodine content is adequately iodised the packaged iodised salt sold in Ghana is capable of preventing IDD upon consumption.

Preference for Brand of Salt

According to responses from questionnaire in this study, Table 63, the choice of salt by respondents for a specific brand of iodised salt depended on its availability, popularity, and iodine content, Table 62.

Table 62: Choice of Brand of Salt and Percentage (%) of Respondents

Reasons	%
No idea	27.0
Contains iodine/certified	10.0
Available/popular/certified	47.0
It is good/nice/cheap	7.0
Contains vitamins	1.0
Very white/pure	1.0
Contains right level of iodine	3.0
Available and pure/long shelf life	1.0
All is salt	1.0

Source: Field Work, Benjamin Bartels, UCC, (2017)

The responses also indicated that Annapurna is the preferred choice by 56% of the respondents as depicted in Table 63.

Table 63: Preferred Brand of Salt and Percentage (%) of Respondents

Brand of salt	Respondents (%)
No idea	28
Annapurna	56
U2	9
Annapurna/U2	6
Master chef	1

Source: Field Work, Benjamin Bartels, UCC, (2017)

This is because it is widely distributed with percentage coverage of 100 %, Table 92. U2 was next with 74% distribution, followed by Concord

plus, Mr. Chef, Cerebos and Lele having 48, 39, 13 and 6.4% respectively. The salt samples with the lowest distributions were Salnova, Ritebrand and Buffalo with 3.2% each, Table 64.

Table 64: Distribution of Iodised Salt Nationwide

Brand	Distribution (%)
Concord Plus	48
Salnova	3.2
Cerebos	13
Buffalo	3.2
Ritebrand	3.2
Annapurna	100
Mr. Chef	39
U2	74
Lele	6.4
Ante Dede	3.2

Source: Field Work, Benjamin Bartels, UCC, (2017)

Unpackaged Iodised Salts

Unpackaged iodised salts are sold in open containers or in polyethyelene sachets as shown in Plate 5.

Plate 5: Samples of Unpackaged Salt

This type of salt is not branded and thus difficult to identify the producers, the source of production or supplier. Compared to the packaged salt they are cheaper, affordable, abundant and available in every market and community in Ghana. Thirty-four (34) samples of such salts were collected from selected markets across the ten regions of Ghana.

Iodine concentration in unpackaged salt

The salt sample from Wa in the Upper West region was found to contain 52 ppm iodine. The Agbogbloshie sample contained 34 ppm iodine whilst the Madina sample had 54 ppm iodine, Table 65.

Table 65: Iodine Content of Unpackaged Salts in Ghana

Region	Town	District	Iodine level, mg/kg
Volta	*Ho*	Ho Municipal	Non-detect
	Aflao	Aflao Municipal	Non-detect
	Blekusu	Ketu South	Non-detect
	Adina	Ketu South	Non-detect
	Anlo-Afiadenyigba	Keta Municipal	Non-detect
	Anloga	Keta Municipal	Non-detect
	Dabala		Non-detect
Northern	*Tamale*	Tamale Municipal	Non-detect
	Daboya	West Gonja	Non-detect
Upper West	*Wa*	Wa Municipal	52.00
Upper East	*Bolgatanga*	Bolgatanga Municipal	Non-detect
Brong Ahafo	*Sunyani*	Sunyani Municipal	Non-detect
Eastern	*Koforidua*	New-Juaben	Non-detect
	Krobo-odumase	Municipal	Non-detect
Ashanti	*Kumasi*	Kumasi Metropolitan	Non-detect
Central	*Cape Coast*	Cape Coast	Non-detect
	(Kotokuraba)	Metropolitan	Non-detect
	Mankessim	Mfantsipim	Non-detect
	Apam	Gomoa	Non-detect
	Agona Swedru	Agona West	Non-detect
	Elmina	KEEA	Non-detect

Table 65: Continued

	Kuntu	Mfantiman	Non-detect
	Kasoa*	Gomoa	
Western	*Takoradi*	Sekondi – Takoradi	Non-detect
	Jarway-Warf	Nzema East	Non-detect
	Axim		Non-detect
Greater	*Accra (Kaneshie)*	Accra Metropolitan	Non-detect
Accra	Tema	Tema Municipal	Non-detect
	Sege	Ada West	Non-detect
	Kassei	Ada East	Non-detect
	Agbogbloshie		34.00
	Makola		Non-detect
	Madina		54.00
	Ashiaman*		Non-detect
	Nima		Non-detect

Source: Laboratory results, Benjamin Bartels, UCC, (2017)

The Agbogbloshie sample satisfy the mandatory limit of 25-50 ppm iodine, with 34 ppm, Table 65, the samples from Wa in the Upper West Region and Madina in the Greater Accra Region exceeded the upper legal limit of 50 ppm, Table 98. The iodine concentrations of salt samples from Upper East, Northern, Brong Ahafo, Eastern, Western, Central, Ashanti and Volta Regions had concentrations too low to be detected (non-detect) as shown in Table 65. The unpackaged salt enjoys high patronage by volunteers as compared to the packaged salt according to this study.

The reasons given by respondents included familiarity with non-iodised salts; worsening of taste of food by iodised salt; high cost of iodised salt; iodine toxicity; and superstition. These reasons determined the behaviourial pattern of respondents (consumers) with respect to choice of salt.

It could be inferred from the reasons that the volunteers were unaware that the unpackaged salts are to be iodised by law, even as they are ignorant about the mandatory salt law. This behaviour if not addressed could contribute to the prevalence of iodine deficiency in the study population, and defeates the purpose for which the law was introduced by the Government of Ghana in 1996.

The other reasons that could explain the relative abundance of uniodated salt on the market are inefficient monitoring of the production, distribution and sale of iodated salt by Food and Drugs Authority of the Ministry of Health (Ghana Nutrition Profiles, 2011). That only 3% of the unpackaged salt investigated contained iodine, the unpackaged salt sold on the market is not adequately iodised and hence incapable of fighting IDD.

Magnesium Content of the Unpackaged Salt

The magnesium content in the salts is considered as impurity, and were found to be ranging from 0.18 to 0.31% as shown in Table 66.

Table 66: Magnesium Content of Unpackaged Salt

Region	Market	Magnesium content , %
Volta	Daboya	0.18
	Aflao	0.21
	Blekusu	0.23
	Adina	0.24
	Anlo-Afiadenyigba	0.26
	Dabala	0.26
	Ho	0.26
	Anloga	0.28
Upper West	Wa	0.19
Upper East	Bolgatanga	0.20
Northern	Tamale	0.21

Table 66: Continued

Region	Market	Magnesium content , %
Western	Jarway-wharf	0.22
	Sekondi	0.22
	Takoradi	0.23
	Axim	0.28
Eastern	Krobo-Odumase	0.22
	Koforidua	0.31
Central	Cape Coast	0.25
	Elmina	0.25
	Apam	0.26
	Kasoa	0.28
	Agona Swedru	0.29
	Mankessim	0.29
Greater Accra	Kaneshie	0.25
	Madina	0.26
	Tema	0.27
	Agbogbloshie	0.28
	Makola	0.28
Brong Ahafo	Sunyani	0.26
Ashanti	Kumasi	0.27

These concentrations are consistent with the results of 0.05 to 0.30% reported by Zimmermann (2009). Such levels of magnesium will cause the salt to absorb moisture and hence affect its iodine stability.

Metal Contents in Salt

Annapurna, U2, and Ante Dede had Pb concentrations of 51, 13.31, and 3.4 ppm respectively, Table 67. These concentrations were higher than the maximum tolerable limit of 2 ppm recommended by Codex, and are also much higher than 0.85, 0.44 and 0.50-1.64 ppm found in earlier studies done in

Turkey, Egypt, Greece, Iran and India by Heshmati et al, (2014), Abdol Majid et al., (2010) and Soylak et al, (2008).

Table 67: Brands of Salt and Concentrations (µg/g) of Metals

Salt	Pb	Fe	Cd	Zn	Al
Concord Plus	0.61	trace	0.20	trace	0.14
Salnova	0.20	trace	0.05	trace	0.20
Cerebos	0.30	trace	0.01	trace	0.16
Buffalo	0.58	trace	trace	trace	0.26
Ritebrand	1.0	trace	trace	trace	0.10
Annapurna	51.0	trace	trace	trace	0.28
Mr. Chef	1.4	0.95	trace	trace	trace
U2	13.31	0.49	trace	trace	trace
Lele	1.4	0.58	trace	trace	trace
Ante Dede	3.4	0.25	trace	trace	trace
Unpacked (Composite)	trace	trace	trace	trace	trace
Codex Limits	2.0	-	0.5	20.	2.0

Source: Laboratory Results, Benjamin Bartels, UCC, (2017)

Trace means below detection limit

On the other hand, Concord Plus, Salnova, Cerebos, Buffalo, Mr. Chef, Lele and Ante Dede had Pb concentrations of 0.61, 0.20, 0.30, 0.58, 1.4 and 1.0 ppm respectively. These were lower than the maximum tolerable limit of 2 ppm recommended by Codex. Such samples when consumed do not pose any health hazards to consumers. On the other hand, Ritebrand has trace (too low to be detected) concentration of Pb making it the most wholesome salt with respect to Pb.

The concentrations of Fe were trace for Concord Plus, Salnova, Cerebos, Buffalo, Mr. Chef, Lele and Ante Dede. This suggests that the equipment and tools that were used for the production of these iodated salts were rust-free. Mr. Chef, U2, Lele and Ante Dede had 0.95, 0.49, 0.58 and 0.25 ppm levels of Fe respectively. Apart from Mr. Chef, the concentrations of Fe in U2, Lele and Ante Dede found in this study is less than the concentrations in packaged salt, 0.69 ppm, found in earlier studies (Hashmati et al., 2014). The composite sample of unpackaged salt, Sample K had trace concentration of Fe. The concentrations of Zn were trace in all the samples including the unpackaged salt.

Buffalo, Ritebrand, Annapurna, Mr. Chef, U2, Lele, Ante Dede and unpacked salt (composite sample) had trace concentrations of Cd whilst Samples Concord Plus, Salnova and Cerebos had Cd concentrations of 0.20, 0.05 and 0.01 ppm respectively below the Codex maximum tolerable limit of 0.5 ppm, Table 67. These concentrations were also less than 0.229 ppm found by Hashmati et al., (2014), and higher than the 0.024 ppm found by Abdol Majid et al., (2010).

With regards to Al, Mr. Chef, U2, Lele, Ante Dede and unpackaged salt had trace concentrations, whilst Concord Plus, Salnova, Cerebos, Buffalo and Ritebrand recorded 0.14, 0.20, 0.16, 0.26 and 0.10 ppm, Table 67. These concentrations were found to be below the codex maximum limit of 2 ppm and also below the 5.82 ppm reported by Ghulam et al., (2007).

Effect of Temperature on Salt Iodine Stability

The stability of the iodine in the salt was found to depend on temperature. It was found out that as the temperature was varied from 30 ^{o}C to

180 °C, Table 68, the stability of the iodine in the salt reduced from 97% to 83.1% was affected resulting in the loss of iodine, respectively form 3.1% to 16.9%, Table 68.

Table 68: Effect of Temperature (ºC) on Iodine Stability of Salt

T °C	Initial concentration of iodine in salt, mg/kg	Volatile iodine by difference, mg/kg	% Loss	% Stability
28	46.00	0.0	0.0	100.0
30	44.61	1.39	3.1	97.0
100	42.40	3.60	7.8	92.2
180	38.04	7.76	16.9	83.1

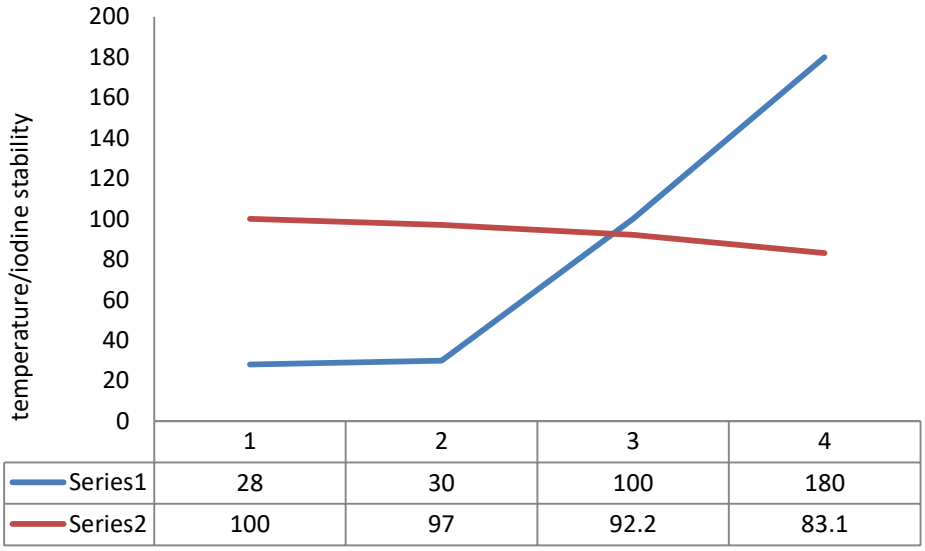
Source: Laboratory Results, Benjamin Bartels, UCC, (2017)

Duration of exposure is 2 hrs

% loss of iodine = $\left(\frac{Volatile\ iodine}{Concentration\ of\ iodine}\right)$ x 100

% stability = 100 - % loss

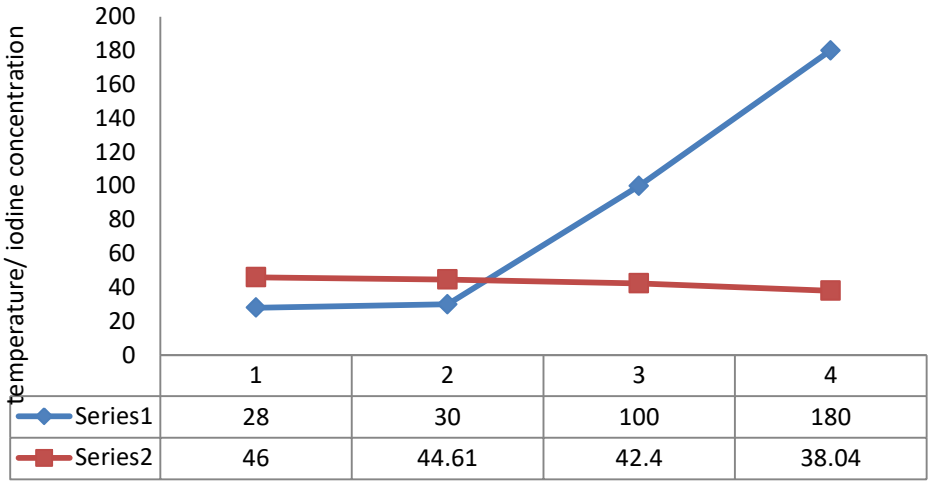
The effect of temperature on iodine stability is shown in Figure 16. As temperature (blue series) increases from 28 to 180ºC, the stability (red series) reduced from 100 to 83.1% as shown in Table 68 resulting in a loss of 25%. There was a strong negative correlation of – 0.99 at 95% sig between temperature and stability of iodine.



	1	2	3	4
Series1	28	30	100	180
Series2	100	97	92.2	83.1

Figure 16: Efect of Temperature on Iodine Stability.

Figure 17 depicts relationship between temperature and iodine concentration.



	1	2	3	4
Series1	28	30	100	180
Series2	46	44.61	42.4	38.04

Figure 17: Temperature and Iodine Concentration.

Pearson's correlation showed also a strong positive correlation of +0.99 found between temperature and loss of iodine. This suggests that temperature and loss of iodine are strongly related and that temperature increases (blue series) with loss of iodine (red series). For example, at 30^{o}C the percentage loss of iodine was 3.1%, and at 100^{o}C the corresponding loss of iodine was 7.8% as shown in Table 68 and Figure 17. The findings are consistent with similar observation by Stephanie, (2014).

It must be emphasised that the key temperatures considered for this study were 30, 100 and 180 ^{o}C, representing ambient condition, cooking (boiling) condition and frying/baking conditions respectively. These are usual temperature conditions that iodaised salt is exposed to by users. At the start of the study, there was no loss of iodine, and therefore had 100% iodine concentration. At 30 ^{o}C, there was a loss of 3.1% iodine, whilst at 100^{o}C and 180^{o}C there were iodine losses of 7.8% and 16.9% respectively, Table 68. It could be inferred that when iodised salt is stored at ambient conditions there will be loss of iodine, and that at higher temperatures like cooking and frying temperatures much of the iodine is lost. According to Diosady et al, (2000), in most chemical reactions involving iodine, high temperatures increase the rates of the reactions that form the elemental iodine and then increase the rate of loss of the iodine.

It could also be inferred that in order to succeed in using iodised salt as a preventive for IDDs in Ghana, the iodated salt must be stored and used at ambient conditions not exceeding 30 ^{o}C considering the significant loss of 8.5% to 16.9% iodine during cooking. Iodised salt should preferably be used as table salt and not as cooking salt.

The stability of the iodine was also found to decrease with time upon exposure to the weather as seen in Table 69. At the start, the iodine concentration in the salt was found to be 50.0 ppm (100% stability), moisture content was 0.01%, and the humidity of the climate within the period was 63%, Table 97, with the magnesium content in the salt found to be 0.30%. At week 12, the moisture content increased to 4.57%, Table 69, with a corresponding iodine reduction of 59.80%.

Table 69: Effect of Humidity, Moisture Content and Magnesium Content on Iodine Stability

Week	Iodine Stability mg/kg	Moisture content,%	Humidity %	Magnesium content,%
0	50.00	0.01	65-60	0.30
1	44.61	0.05	62-63	0.30
2	42.30	0.12	68-80	0.30
3	41.24	0.01	50-55	0.30
4	38.07	0.32	53-50	0.30
5	36.06	1.03	40-48	0.30
6	32.13	2.50	89-90	0.30
7	31.75	0.02	60-64	0.30
8	29.85	0.16	70-72	0.30
9	28.57	0.05	70-74	0.30
10	25.92	0.11	70-72	0.30
11	22.22	0.05	60-74	0.30
12	29.90	28.57	70-74	0.30

Source: Laboratory Results, Benjamin Bartels, UCC, (2017).

This high moisture and a corresponding salt iodine loss were influenced by the fairly high relative humidity, Table 69, because the iodine can be oxidised to elemental iodine by oxygen and moisture causing the iodine to be rapidly lost to the atmosphere through diffusion according to Diosady et al, (2000). Again, the high salt magnesium content of 0.30% in the composite salt contributed significantly to the loss, because it is a hygroscopic impurity, its effect is to act as a reaction medium to decompose the added iodine the pH

of the condensed moisture on the salt and eventually affect the stability of the salt according to Diosady et al., (2000). For instance at Week 6 it rained heavily so the weather was humid, with a relative humidity of 89-90 %, that increased the moisture content of the salt to 2.50%, Table 69. The stability therefore depended on the relative humidity. Pearson's correlation, showed a strong negative correlation of –0.99 at 95% significance between duration of exposure and stability of iodine.

This is evidenced by the fact that the longer the exposure (12 weeks) of the salt the lesser the stability and the greater the loss (4.57%) of the iodine. Again, a weak negative Pearson's correlation, of -0.014 was obtained for duration of exposure and moisture content. This implies that moisture in the salt was affected by the length of exposure to the weather. There was also a very weak negative correlation of -0.056 between moisture content and iodine stability and that as moisture increases iodine stability reduces.

Bacteriological Studies

Apart from Annapurna, the rest of the salt recorded CFU of zero, an indication that no colony forming units (CFU), Plate 6, were found and that microorganisms were absent. This is an indication that the salt samples were produced in a cleaner and hygienic environment which is void of anthropogenic activities. This finding confirms previous studies that salt has the ability to control microbial growth according to Jayetisser & Gunathilaka, (2001).

On the other hand, microbes were observed in the Annapurna salt, CFU/ml of 2.0, Plate 5.

Plate 6: Presence of Microbes

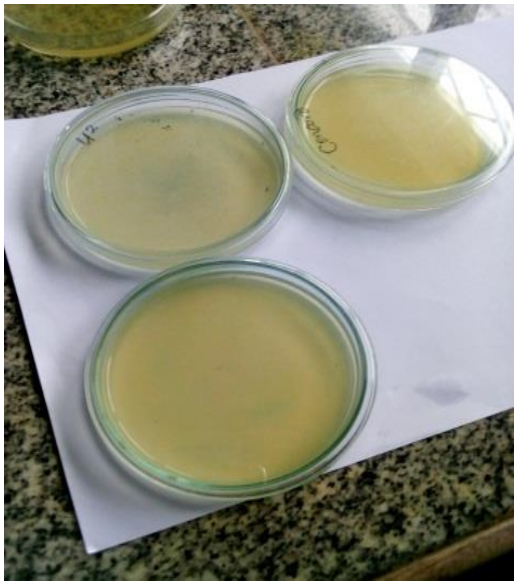


Plate 7: Absence of Microbes

The colonies found in Annapurna may have arisen during packaging inspite of the high salinity environment. This collaborates the findings by Mona, (2014) and Eviatar et al., (2014) that microbes can survive in high salinity environment and could cope 'actively' with its extreme environment .

Behavioural Pattern Responsible for IDDs in the Study Populations

A total of four - hundred and seventy-one (471) questionnaires were administered to patients who have been diagnosed with goiter; to traders (Market); staff and pupils of Hillcrest schools and undergraduates of UCC. The purpose was to seek information about their behaviour towards iodised salt and iodine deficiency. Apart from the iodine status and the mandatory salt iodisation law, the other predictors considered for this study have been applied Wiersinga, (2013) and Dalia et al., (2017) to determine the prevalence of IDD in their respective studies. The summary of the survey responses (excluding pupils) is shown in Table 70.

Table 70: Summary of Survey Responses of Respondents

Predictors	Hospital	Market	Staff	Undergraduates
Number of respondents, n	70	12	38	179
Gender (female) / %	89	58	67	26
Gender (male) / %	11	42	33	74
Age range / yrs	10-79	20-69	20-69	19-39
Literacy rate / %	86	100	100	100
Consumption of iodised salt / %	89	100	97	97
Knowledge about iodine deficiency / %	75	50	54	75
Iodine status test(not tested) / %	96	100	100	91
Knowledge about the law / %	0	0	0.1	21
Consumption of goitrogens (raw cabbage) / %	76	75	95	91
Consumption of goitrogens (millet) / %	70	92	85	70
Consumption of goitrogens (peas)/%	59	83	51	56
Consumption of goitrogens (smoking)/%	17	33	0	2
Consumption of goitrogens(drinking water)/%	90	91	94	99
Consumption of goitrogens(soyabean)/%	43	50	69	79

Source: Field work, Benjamin Bartels, UCC, (2017).

Hospital Survey

The survey involved a total of 70 Patients who have diagnosed with thyroid cases. The survey was carried out in a period of 6 mths. The summary of survey responses is presented in Table 70.

Gender

Eighty-nine percent (89%) of the total respondents diagnosed with the disease (patients) were found to be females in this study. Such incidence of higher goitrous females than males has also been reported by Boelgert, (2004), Dalia et al., (2017), and Wiersinga, (2013). The prevalence has been attributed to Hashimoto's hyperthyroidism an autoimmune disease which is in general more common in women.

Age

Table 71 shows age range, number of patients and percent goiter.

Table 71: Age of Patients, number of patients and percent goitre Incidence at the Cape Coast Teaching Hospital

Range of ages (yrs)	Number of patients	Percent goitre, %
10-19	2	3
20-29	16	23
30-39	17	24
40-49	22	31
50-59	10	14
60-69	2	3
70-79	1	1

Source: Field work, Benjamin Bartels, UCC, 2017

The class, 10-19 yrs considered by WHO as the school-going age and most vulnerable (WHO, 2004) had 3% being diagnosed with a thyroid disorder as shown in Table 71. These patients could suffer low IQ and severe brain damage, poor academic performance and in extreme cases cretinism according to Boelgert et al, (2004). Though 3% might appear insignificant, yet

the effect of the disorder on even one child is grievous to the nation since they form the foundation of future workforce and leadership. This finding is contrary to that found in Sudan, where a study about iodine status of school going-age children indicated a higher prevalence of goitre (Medani et al, 2013).

The class of 30-39 yrs formed 24 % of the patients, Table 71. This finding is contrary to the relatively higher percentage of goiter prevalence in this group found by. This class generally constitutes the threshold and energetic working group and that such a percentage diagnosed with thyroid disorder could affect the productivity of Ghana. The class of 40-49 recorded the highest percentage of 31 %, of the Patients. This finding agrees with the findings of Dalia et al., (2017) who reported that goiters are more common in people within this group and confirmed smoking, diets and gender as factors associated with the prevalence of thyroid disorders in middle-aged subjects as found in this study.

The group of 50-59 yrs had 14% prevalence rate, Table 71. This finding is contrary to the observation by Diez, (2005) who reported that goiters are common in such older people. The 60-69 class was 3%, and the 70-79 class had 1%. This is expected because at these ages metabolic processes in the body are reduced drastically.

On the whole, the study revealed a characteristic pattern of the disorder with respect to age. As the age increases, the prevalence increases from 3% and peaked at the 40-49 yrs at 51% and then fall sharply at 50-59 yrs to 14% and then assumed a steady decline to 70-79 yrs at 1%, Table 71. This observation confirmed that age is one of the risk factors of goiter and that

thyroid disease affects all age groups as reported by Thyroid Cancer, (2016) and it is more pronounced among the 40-49 yrs.

Educational Background of Patients

Literate patients constituted 86%, Table 71. This finding was contrary to the findings by Khan & Siddiqui, (2003) who found out that the prevalence of goitre in Jamalpar District of Bangladesh showed that a maximum of 53% of the respondents were illiterate. In this study, the illiterate patients showed their ignorance about the benefits of iodised salt, its storage and cost, and also the causes of IDD as depicted in Appendices S – U. This is rather in agreement with Khan and Siddiqui, (2003) who found out that illiterate patients mentioned high cost of iodised salt, and showed their ignorance in its benefits.

Again, Wolka et al., (2014), Guerdeep et al., (2013) and Veena et al., (2015) have found out that illiterate parents could impact negatively on their children as children of such parents perform poorly at school, and that literacy/illiteracy status has a bearing on the goitre status.

Knowledge about iodised salt / Consumption of iodised salt

It was found out that 89% consume iodised salt, Table 70. This is in agreement with findings by Khan & Siddiqui, (2003) and Fisher et al, (2011) who respectively reported higher percentages of consumers. This observation confirms that in countries that have implemented programmes to eliminate IDD like Ghana, there is still a smaller percentage that has not used iodised salt yet.

Benefits of Iodised Salt

Sixty-five percent (75%) of the respondents indicated that it prevents IDD by providing iodine to the body, Appendix T. On the other hand, 34% had no idea about the benefits they were getting from the salt, this collaborates the findings of Khan & Siddiqui, (2003). Appendix V, showed that a total of 73% use it regularly. The study also showed that 46% of the respondents store the salt properly when in use, Appendix S.

According to Dasgupta et al., (2008), after opening the container of the iodised salt the amount of the iodine that stays in the salt for eventual consumption reduces if not stored properly, and this presupposes that the 54% that did not store the salt properly during use would consume reduced amount of iodine; this might contribute to their being diagnosed with thyroid disease. Appendix W, shows that 73% of the respondents were found to have a high preference for iodised salt. The reasons for the preference, Appendix T, included health benefits and prevention of goiter.

The non-iodised salt consumers, Appendix X, gave reasons such as being conversant with it, non-availability of the salt in their locations, cost and the tendency of iodised salt to impart unusual taste to meals. This is in accordance with the finding of Fisher et al., (2011) who reported that iodised salt makes food taste bitter, and was not locally available for purchase according to his respondents. The most preferred iodised salt was found to be Annapurna, Appendix Y. Reasons given for their preference, Appendix T, included availability, popularity, and presence of iodine.

In Appendix Z, 77% of the respondents have used iodised salt for less than 10 yrs, 7% have used it more than 10 yrs, with 3% having consumed it

since childhood. The study thus confirms the findings of Kotwal, (2006) who reported that goitre was found in households that had been consuming iodised salt for more than 10 yrs and that one could still be consuming iodised salt and develop IDD as found in this study.

Knowledge about Iodine Deficiency Disorders

Fifty-six (56%) percent correctly answered as a growth, swelling or expansion of the thyroid gland due to lack of iodine, Table 33, whilst the rest showed their ignorance.

Iodine Status

Ninety-six percent (96%) as shown in Table 70, of the patients did not know that it is possible to check their iodine status, and therefore did not know whether they were iodine deficient or not, and as such would not have any reason to regulate their iodine intake.

The Mandatory Salt Iodisation Law

One hundred percent (100%) as portrayed in Table 70, of the respondents are ignorant about the mandatory salt iodised law. This could be lack of publicity about the law on the part of state agencies that are mandated to do so.

Knowledge about Goitrogens (Nutrition)

The selected predictors were raw water, cabbage, soyabean products, millet products, tobacco products, and peas, Table 14. These contain certain chemical compounds called goitrogens consumption of which prevent the uptake of iodine by the thyroid gland (Spheres of Life, 2010). Seventy -Six percent (76%) of the respondents were found to eat cabbage, Table 70. This could be the reason why they have been diagnosed with goiter since cabbage

contains goitrogens namely goitrin and thiooxazolidones (Environmental Health Perspectives, 2010). These goitrogens prevent uptake of iodide into the thyroid gland. Its activity is however destroyed when boiled before eaten (Stephanie, 2014).

Another goitrogen, genistein found in soy foods, is suspected to interfere with a key enzyme in the thyroid gland, throxine peroxidase. 43% of the patients were found to consume soy foods, Table 70. Seventy percent (70%) of the respondents, has preference for millet, this implies that goitrogens from millet could also be responsible for IDD. Millet provides another source of goitrogen in our diets. Seventeen percent (17%), were found to be smokers. Such patients are exposed to cyanide which is a goitrogen in tobacco and has confirmed that smoking is one of the predictors of thyroid disorders (Dalia et al., 2017; Wiersinga, 2013). Peas contain goitrogen called arachidoside that prevents the uptake of iodine. 59% of the respondents consume peas.

Univariate, bivariate and multivariate analysis were used to analyse the responses of the patients. The Cramer's V was used to determine the extent of the association between the variables.

Univariate analysis

The consumption of goitrogenous foods correlated positively (Pearson $chi^2 = 32.0869$, $p = 0.000$) with being diagnosed with goitre. Cramer's V of 0.3575 suggests a very strong and desirable association, Appendix ZA.The frequency of use of iodised salt has an association with goitre, Pearson's chi2 gave 9.3268, $p = 0.009$, and with cramer's V of 0.1855 as found in Appendix

ZA. Though the Cramer's V hinted on a weak association, not using the salt often could lead to goitre.

Age as shown in Appendix ZC, of the respondents correlated strongly with being diagnosed with goitre (Pearson chi^2 96.4862 at p = 0.000) with a much stronger Cramer's V of 0.5945. The age range of 70-79 yrs is most vulnerable, followed by 40-49 yrs, with the least being 10-19 yrs.

There is also a strong association between goitre and educational status, Appendix ZD (Pearson chi^2 gave 109.0332 at p = 0.000) with Cramer's V of 0.6414. Thus, a respondent with no-formal-education has 100% likelihood of being diagnosed with goiter. On the other hand the respondent with tertiary education status is 9% likely to be diagnosed with goitre.This agrees with the findings of Khan & Siddiqui, (2003).

Bivariate Analysis

Negative log-log bivariate regression model was used in predicting diagnosed goitre. There is 12 % likelihood, p = 0.016, of being diagnosed with goiter when goitrogenous foods are consumed, Appendix ZK.This observation agrees with the findings of Dalia et al., (2017). The ages from 20-79 yrs have high probability, p = 0.000 of being diagnosed with goitre, Appendix ZF, than those within 10-19 yrs. This observation has been made by Atul Kotwal, (2006). The females are 74% (1-0.2659 = 0.74 x 100) more likely to be diagnosed with goitre than males 26%, Appendix ZH. 85% of the respondent knew about iodated salt, Appendix ZI and less likely to be diagnosed with goitre at p = 0.009. 44% of respondents (most often) were less likely to be diagnosed than the 23% who are more likely to be diagnosed with goitre, Appendix ZJ.

Multivariate Analysis

Three models were formed to determine the cumulative effect of the predictors. In model 1, a difference of zero at p = 0.892 indicates that nutrition mediate well in goiter diagnosis, but depends on the age and the gender of the patient. For instance the p values are < 0.05. Again, when model 1 is combined with knowledge, there is 2% likelihood of goiter with p values less than 0.05, Appendix ZK.

The Non-Goitrous Respondents

Market Survey

50% had no knowledge about iodine deficiency disorders, and 83% did not know that consuming iodised salt cures goitre, as shown in Table 70 and Appendix ZL. All respondents were ignorant about checking their iodine status, and might not be aware whether they are iodine deficient or not. Moreover, none of the respondents knew about the law as shown in, Table 70. Goitrogenous consumption was very high among the respondents as shown in Table 70.

Education Institutional Survey

The knowledge of the staff (54%) about iodine deficiency disorders, Table 50, indicated that it is a disease that causes the expansion of the thyroid gland due to lack of iodine. None (100%) had checked their status, Table 71. Such a high percentage does not know whether they are iodine sufficient or not. The respondents' knowledge about the existence of the law was poor, as only 36% knew about the law, Table 70. They also had preference for goitrogenous foods. 95% eat cabbage, Table 70, whilst 69% consume soy food, with 85% consume millet, Appendix ZM. Again, 51% consume peas.

Thus the respondent's desire for goitrogenous foods could result in IDDs.

Preparatory School Pupils

A total number of 172 pupils participated. The questionnaire sought information about consumption iodised salt and raw cabbage and how they affect their academic performance. The Mean and the Standard Deviations of the dependent variable (Academic performance) and the independent variable are shown in Table 68. Academic performance recorded 3.20 ± 0.131; raw cabbage 1.72 ± 0.453 and iodised salt is 1.83 ± 0.381.

Table 70 shows the output of the ANOVA analysis and whether there is a statistically significance difference between the group means. The significance value of 0.968 for cooked or raw cabbage consumption is above the value of p = 0.05, therefore there is no significant statistical difference in the consumption of raw cabbage and academic performance. Thus from a 1 – way ANOVA, Appendix 140, results indicated that the means of the two conditions are equal F (1,170) = 0.002, p= 0.968.

Similarly, there is no significant statistical difference in the consumption of iodated salt on academic performance. Thus the two conditions are also equal, F (2,169) = 1.171, p = 0.313, Table 70. These mean that the predictors or the independent variables consumption of cabbage and iodised salt have equal or somewhat equal impact on the dependent variable of academic performance. Pearson's correlation test was done to determine the correlation between academic performance and the consumption of cabbage either cooked or raw. The result, Table 44, indicated that there was a weak negative correlation between academic performance and cabbage consumption given r = -0.003 and p = 0.484. This suggests that as the consumption of raw

cabbage reduces, academic performance increases and vice versa. This is because raw cabbage contains goitrogens called goitrins and oxazolidenes that prevent the uptake of iodine, as the consumption reduces the effect of the goitrogens on the thyroid gland also reduces and thereby improves the IQ. This collaborates the findings of Kotwal et al., (2006), Guttinkoda, (2014), and Hess, (2010).

There was however a positive correlation though very weak, between academic performance and iodated salt consumption with r = 0.117 at P = 0.063. This suggests that as consumption of iodised salt increases, academic performance increases as well, and vice versa. This observation has been highlighted by Fisher et al., (2011) and Abdel Monim et al., (2011)

Tertiary Institution Undergraduates

One hundred and seventy-nine (179) undergraduates form the University of Cape Coast Ghana, participated. Their knowledge about iodised salt 97%,Table 70 and 86% knew that it is a disease that causes the swelling of the neck due to lack of iodine. 91%, Table 70, of the respondents have not checked their iodine status. 79%, Table 70 did not know that a law has been passed to mandate the iodisation of all edible salt for consumption by humans and animals in Ghana. Such a high rate of ignorance could increase IDD in the country. In this study, 91% of respondents were found to consume cabbage; 79% that consume soya food, Table 70. Again, millet which also are goitrogenic had 70% of the respondents consuming it. Cigarette smoking results in ingestion of goitrogens called thiocyanate. Two percent (2%) admitted smoking cigarette. Peas which are also goitrogens were consumed by 56 % of the respondents, Table 70. Such levels of consumption could lead to

IDDs.

Chapter Summary

The minimum, maximum and the median urinary iodine concentrations obtained for the study populations by the analytical methods are presented in Table 72. Comparative studies of the results by Horwitz ratio indicated that the results by the novel method are most reliable, with ratio of 0.12. Sandell-Kolthoff and Classical titration methods had ratio of 0.5 and 1.4 respctively.

Table 72: Summary of Urinary Iodine Concentrations, (ppm) of Populations

Population	Classical	titration	method	Sandell-	Kolthoff	method	Novel	method	
	Minimum	Maximum	Median	Minimum	Maximum	Median	Minimum	Maximum	Median
Staff	ND	183.38	30.21	40.46	341.87	77.69	143.46	4730.80	863.03
Pupils	8.48	190.80	43.46	50.02	306.22	102.08	123.92	1219.20	416.56
Undergraduates	11.44	106.56	48.31	33.72	216.70	106.74	114.40	988.0	440.40
Household	10.58	99.60	31.74	40.28	93.78	70.28	152.40	406.40	264.16

Source: Laboratory Results, Benjamin Bartels, UCC, (2017)

ND: non-detect

Discriminant analysis showed that the novel method classified the population correctly with 77%. Percentage recovery was 125%, and the procedure for the method was optimised and validated. The average concentration of iodine found in Concord plus was 29.0±0.003 ppm; Salnova, 40.0±0.00 ppm; and Cerebos, 34.0±0.01 ppm. The rest were Lele and Ante Dede with 43.0±0.02 and 47.0±0.01 ppm respectively. The average concentration of iodine in Buffalo was 10.0±0.04 ppm, and Ritebrand being non-detectwhich was below the minimum legal limit of 25 ppm. However, Mr. Chef, Annapurna and U2 were respectively 51.0±0.004, 50.0±0.02 and 51.0±0.02 ppm.

The unpackaged salt samples were non-detect except samples from Agbogbloshie and Wa with concentrations of 34 and 52 ppm respectively. The stability of the iodine in the salt was found to depend on temperature, moisture and magnesium impurity. Annapuurna, U2 and Ante Dede had Pb concentrations of 51.0, 13.31, and 3.4 ppm respectively. These were higher than the Codex limit of 2 ppm. Concord plus, Salnova, Cerebos, Buffalo, Mr. Chef, Lele and Ante Dede had Pb concentrations of 0.61, 0.20, 0.30, 0.58, 1.4 and 1.0 ppm respectively. These were lower than the maximum tolerable limit of 2 ppm. The concentrations of Fe were trace for Concord plus, Salnova, Cerebos, Buffalo, Rite brand and Annapurna.

Mr. Chef, U2, Lele and Ante Dede had 0.90, 0.49, 0.58 and 0.25 ppm of Fe respectively. Unpackaged salt (composite sample) had trace concentration of Fe. The concentrations of Zn were trace in all the samples including the unpackaged salt (composite sample). Buffalo, Ritebrand, Annapurna, Mr. Chef, U2, Lele, Ante Dede and unpackaged salt (composite

sample) had trace concentrations of Cd whilst Concord plus, Salnova and Cerebos had Cd concentrations of 0.20, 0.05 and 0.01 ppm respectively below the Codex limit of 0.5 ppm. The concentrations of Al were trace for Mr. Chef, U2, Lele, Ante Dede and unpackaged salt (composite sample) and 0.14, 0.20, 0.16, 0.26 and 0.10 ppm for Concord plus, Salnova, Cerebos, Buffalo and Ritebrand respectively which were above the Codex limit of 2 ppm.

Bacteriological studies revealed that Annapurna contained microbes. It recorded TVC of 2.0 CFU/mL.

Univariate analysis of the Patients' questionnaire showed that the consumption of goitrogenous foods, age, educational status and the frequency of use of iodised salt correlated positively with being diagnosed with goitre. The bivariate analysis showed that there is likelihood of being diagnosed with goiter when goitrogenous foods are consumed and that ages between 20-79 yrs have high probability ($p = 0.000$) of being diagnosed with goitre than those with ages between 10-19 yrs. It also showed that the females are more likely to be diagnosed with goitre than males. Multivariate analysis (Model 1: goitrogenous foods + biosocial vs goiter; Model 2: education and knowledge vs goiter; Model 3: behaviourial vs goiter) indicated that nutrition mediate well in goiter diagnosis.

CHAPTER FIVE

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Overview

The study sought to find the causes of iodine deficiency in populations that reside in the Central region of Ghana. The objectives were to analyse the concentrations of iodine in the salt consumed and the urine excreted by the population. The methodology that was used to achieve the objectives included empirical methods and questionnaire analysis.

Extensive literature which included research papers and publications on the subject, its methodology and previous results were reviewed. These provided a valuable insight for the study. The study covered both field and laboratory work. The urine and salt samples were collected, and questionnaires issued to volunteers from the field, same were analysed in the laboratory.

The results showed that the populations were largely iodine sufficient. The behavioural patterns that could cause the deficiency were found to be high consumption of goitrogens, ignorance of the iodisation law, and the test for iodine status. The packaged salt was found to contain adequate iodine as compared to the unpackaged salt.

The objectives set helped to highlight the potential causes that could cause the deficiency in the study populations. The findings indicated the need to intensify the monitoring of the quality of iodised salt in Ghana. It could also help policy makers to review policies regarding the elimination of IDD.

The major setback of the research methodology was the lack of technologically advanced instruments for the analysis.

Summary

The main objective of this study is to determine the causes of IDDs in the study populations. This was done by determining the concentrations of iodine in iodised salt on the Ghanaian markets; and the iodine concentrations in the urine samples of the populations. Questionnare was also administered to determine the behavioural pattern of the study population.

The specific objectives, however, were to:

1. Analyse the concentrations of human urinary iodine of the population to assess the median urinary iodine concentration;
2. Analyse the concentrations of iodine in iodised salt and the factors responsible for its compliance or non-compliance with the legal limit set by the Ghana Standards Authority (GSA) to prevent IDD;
3. Administer questionnaire to volunteers whose responses were used to highlight the behavioural patterns responsible for IDDs in the study populations.

The study was guided by two hypotheses based on the levels of iodine in the iodised salt and in urine. With respect to the level of iodine in iodised salt, the hypothesis was that the iodised salts sold in Ghana and used for the study contained 25-50 ppm iodine set by GSA. With regards to the urine the hypothesis is that the population is iodine sufficient with a range of 100-199 ppm or 400-649 ppm. The research approach adopted a blend of descriptive and experimental designs. The descriptive design involved the use of questionnaire to gather information about the pattern of behaviour of the respondents towards eliminating IDD in Ghana. The experimental design, on the other hand, involved laboratory analysis of the human urine samples and

the iodated salt samples to collect empirical data. The sampling included the collection of 1,048 urine samples, and 250 iodised salt samples, and 471 questionnaires to volunteers.

The analyses of urinary iodine were done using approved methods and a proposed method. The iodine content in the iodised salt was also determined with WHO approved method. The responses of the questionnaire were analysed by SPSS version 22 and STATA. The median concentrations for the staff, pupils, undergraduates and household were 30.21, 43.46, 48.31 and 31.74 ppm by the classical titration method. The Sandell-Kolthoff method produced median values of 77.69, 102.08, 106.74, and 70.28 ppm respectively for staff, pupils, undergraduates and household. The novel method recorded 863.03, 416.56, 440.40 and 264.16 ppm for staff, pupils, undergraduates and household respectively.

Most of the packaged salt was found to be adequately iodised, whilst the unpackaged salt was mostly uniodised. Factors that were found to cause iodine loss in this study included temperature, moisture and magnesium impurity. Microbes were also found in Annapurna salt. Again, there were high levels of lead in Annapurna and U2.

Conclusions

The Novel method was most suitable for the determination of the urinary iodine concentrations (UICs) of the study populations. The UIC of the study populations indicated that the populations were largely satisfactorily iodine sufficient, and that IDDs could be absent. The bahaviourial patterns of the study populations that could erode the gains were high consumption of

goitrogens, ignorance about the mandatory iodated salt law, and ignorance about the test for iodine status.

Analysis of iodine in the packaged iodised salt showed that majority of the samples were adequately iodised whilst others do not contain iodine at all though branded as being iodised. The unpackaged salt had majority of the samples not iodised. The stability studies of the iodated salt indicated that moisture, and magnesium content in the salt contributed significantly to iodine loss in the salt samples. Temperature was also found to have profound effect on the stability of iodine in the salt.

The most preferred iodised salt sample in the study, Annapurna, (a composite sample) was found to contain high level of Pb above the CODEX tolerable limit, making consumption of it a health hazard. However, Fe, Cd and Zn concentrations were trace and therefore do not pose any health hazards on consumption. The Al concentrations were also found to be within the safe limit given by codex. The unpackaged salt (composite sample) was also found not to contain Pb.

Microbiological studies also indicated that Annapurna contained microbes.

Recommendations

The following recommendations are being made:

1. The monitoring of the producers of iodised salt must intensify to ensure that right level of iodine is put in the salt, and that they are produced under hygienic conditions.
2. The mandatory law should be publicised, this will ensure the right of consumers to probe the iodine content of iodised salt.

3. Distributors and retailers of iodised salt must do so under right conditions to avoid iodine losses.
4. Iodised salt must rather be used as table salt instead of cooking salt to minimize iodine losses, otherwise the concentration of the iodine must be increased to compensate for the loss.
5. The clinical test for iodine status must be enlisted on National Health Insurance, to compel the populace to undertake the test.

REFERENCES

Abdol Majid, C., Farzad, K & Faeizy. N. (2010). Heavy Metals Contamination of Table Salt Consumed in Iran. *Iranian Journal of Pharmaceutical Research 9(2): 129-132.*

Agency for Toxic Substances and Disease Registry. (2000a). *Hanford infant mortality and fetal death analysis* 1940-1952. Atlanta, GA: U.S. Department of Health and Human Services. PB2000105892.

Agency for Toxic Substances and Disease Registry. (2000b). *Toxicological profile for polychlorinated biphenyls,* Atlanta, GA.

Agency for Toxic Substances and Diseases Registry (2006). *Toxicological profile for cyanide.* Atlanta GA: US Department of Health and Human Services, Public Health Service.

Agency for Toxic Substances and Diseases Registry (2004 a). *Guidance manual for the assessment of joint toxic action of chemical mixtures.* Atlanta GA: US Department of Health and Human Services, Public Health Service.

Allen L ., (Ed.) (2006). *Guidelines on food fortification with micronutrients*. Geneva, World Health Organization and Food and Agricultural Organization of the United Nations.

American College of Medical Toxicology; American Academy of Clinical Toxicology (2013). "*Five Things Physicians and Patients Should Question", Choosing Wisely:* an initiative of the ABIM Foundation, American College of Medical Toxicology and American Academy of Clinical Toxicology.

Andersen, S., Karnisholt, J., Pederson, K. M. & Laurberg, P. (2007). Reliability of studies of iodine intake, and recommendations for number of samples in groups and in individuals. *Br. Nutr 99*: 813 – 818.

Anderson, M. V. & Zimmernarn, M. B. (2012). Global Iodine Status in 2011 and trends over the past decade. 744 – 750.

Asibey-Berko, E. & Armah, J. G. (2007). *Biological impact assessment study on salt iodisation programe in Ghana (Jirapa and Bongo sentinel districts).* Accra: UNICEF.

Babulai, R., Parimal, P. & Ghosh, P. K. (2010). Determination of iodide and iodate in edible salt by ion chromatography with integrated amperometric detection. *Food Chem 123:* 529 – 534.

Bath, S. C. & Rayman, P. M. (2015). A review of the iodine status of a UK pregnant women and its implications for the offspring. *Environ Geochem Health 37(4)*: 619 – 629.

Bhagat, P. R., Pandey, A. K., Acharya, R., Natarajan, V., Rajurkar, N. S. & Reddy AVR (2008). Molecular iodine selective membrane for iodate determination in salt sampels: chemical amplification and preconcentration. *Anal Bioanal Chem 391:* 1081 – 1089.

Braverman, L. E. & Utiger, R. D., (Eds) (2000). *Werner and Ingbar's: The thyroid: A fundamental and clinical text.* (8th ed). New York, NY: Lippincott Williams and Wilkins.

Buxton, C. & Baguune, B. (2012). Knowledge and practices of people in Bia District, Ghana, with regard to iodine deficiency disorders and intake of iodized salt. *Archives of public Health 70*: 5.

Byeong, C. A. (2011). "Physiologic and false positive pathologic uptakes on radioiodine whole body scan. *In Tech*, Europe.

Center for Drug Evaluation and Research, FDA, US (2011).

Chirawurah, D., Apanga, S. & Addah, J. (2015). Assessing Iodized Salt use in rural Northern Ghana: A mixed method approach, *Food and public Health, 5* (3), 70 – 76.

Dasgupta, P. K., Dyke, J. V. & Kirk, A. B. (2006). Perchlorate in the United States. Analysis of relative source Contributions to the food chain. *Environmental Science & Technology*, 40: 6608 – 6614 (PubMed).

Dasgupta, P. K., Kirk, A. B. & Dyke J. V. (2008). Intake of iodine and perchlorate and excretion and excretion in human milk. *Environmental Science Technology 42*: 8115 – 8121 (PubMed).

Dasgupta, M., Jain, A. & Verma, K. K. (2004). Single drop microextraction or solid phase microextraction-gas cheromatography-mass spectrometry for the determination of iodine in pharmaceuticals, iodized salt powder and vegetables involving conversation into 4 – iodo-N, N-dimethylanine. *J. Chromatogr A. 1023*: 33 – 39.

Dalia, D., Petkeviciene, J & Klumbiene, J. (2007). Factors Associated with the prevalence of thyroid nodules and Goiter in Middle-Aged Euthyroid Subjects. *International Journal of Endocrinology*, *2017*. 8.

De Benoist, C., & Graeme, A. (2002). Eliminating iodine deficiency disorders: Bulletin of the World Health Organizaiton: *the international Journal of Public Helath, 80*(5): 341.

Delange, F. (2000). Endemic cretinism. In: Braverman LE, Utiger RD, (Eds). *The thyroid. A fundamental and clinical text*. Philadelphia, Lippincott, 743–754.

Dietary iodine (2010). Why are so many mothers not getting enough? *Spheres of Life: Environmental Health Perspectives*. 118(10): A438-A442.

Diez, T. T. & Iglesias, P. (2005). Spontaneous subclinical hypothyroidism in patients older than 55 years: an analysis of natural course and risk factors for the development of overt thyroid failure. *J. Clin Endocrinol Metab 89*: 4890 – 7.

Dunn, J. T. (2001). Correcting Iodine Deficiency is more than just spreading around a lot of iodine. *THYROID 11(4):* 363 – 364.

Eastman, J. C. & Zimmermann, M. B. (2018). *The iodine deficiency disorders* De Groot.

Eicher, T. & Avery, E. (2005). Toxic encephalopathies. *Neurol Clin. 31*:353-76.

Eviatar, N., Grigoriev, I. & Gerhard, R. (2014). *Salt Needed:* Tolerance lessons from a Dead Sea fungus Nature Communications.

Fan, L., Tan L., Chen, Y., Zhu, M., Wang K. (2018). Investigation on the factors that influence the prevalence of thyroid nodules in adults in Tianjian, China. *Journal of Trace Elements in Medicine and Biology 50: 537-542.*

FDA (2011). *Centre for Drug Evaluation and Research*. US.

Fisher, J., Tran, T., Biggs, B., & Dwyer T. (2011). Iodine status in late pregnancy and psychosocial determinants of iodized salt use in rural northern Viet Nam. *Buletin of the World Health Organization 89*: 813 – 820.

Gbadebo, A. M, & Nwufoh, C. O. (2010). Iodine Concentrations in blood and urine samples of goitre and non-goitre patients in parts of Ogun State, southwestern Nigeria. *Journal of Chemical exploration 107*(2): 169 – 174.

USAID (2011). *Ghana: Nutrition Profiles.* USAID Ghana Nutrition Data DHS 2008-2014.

Gupta, M., Pillai, A. K. K. V., Singh, A., Jain, A., & Verma, K. K. (2011). Salt assisted liquid-liquid microextraction for the determination of iodine in table slat by high-performance liquid chromatography-diode array detection. *Food Chem 124*: 1741 – 1746.

Guttikonda, K., Travers, C. A., Lewis, P. R. & Boyages, S. (2003). *Iodine deficiency in Unban primary school children: a cross-sectional analysis 179:* 346 – 348.

Harvard Heart Letter (2011). *Reducing salt intake won't make you iodine deficient*. Harvard Health Publishing, vol.1, Harvard Medical School, U.K.

Hays, M. T. (2001). Estimation of total body iodine content in normal young men. *Thyroid 11(7*):671-675.

Hess, S. G. (2010). The impact of common micronutrient deficiencies on iodine and thyroid metabolism: The evidence of from human studies. *Best Pract Endocrin of Metab*. *24(1):* 117-132 [pubmedabstract].

Hormann, R. (2005). *Schildrusenkrankheiten. ABW - Wissenschaft Sverlag*, 4, Auflage, Seite 15 – 37.

Horwitz, W. & Albert, R. J. (2006). The Horwitz Ratio (HorRAt): A useful Index of Method performance with Respect to precision Assoc. *off Anal. Chem 89(4)*: 1095 – 1109.

Hsu, P. P. (2002). Natural medicines comprehensive Database. *J Med Libr Assoc*. *90*(1): 114.

ICCIDD, (2000). *Iodine Status worldwide*. *World Health Organization Global Database on Iodine Deficiency*, Geneva.

ICRP. (2001). *The ICRP database of dose coefficients: Workers and members of the public.* Version 2.01. Elsevier Science Ltd. International Commission on Radiological Protection.

Druz. R (2016). In Excess, The Role of Iodine in Hashimoto's Disease and thyroid Cancer. *Restorative Medicine Digest*. The Association for the Advancement of Restorative Medicine, summer, 2016.

Ingrid, Koo (2018). Using salt as a food Preservative: Misconceptions about Preserving Foods with salts: *Very well Health.*

Institute of Medicine, Food and Nutrition Board. (2001). *Dietary reference intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nicket, Silicon, Vanadium and Zinc.* Washington, DC: National Academic, Press.

Iodine Status Worldwide (2004). *WHO Global Database on Iodine Deficiency.* Bruno de Benoist; Anderson M; Egli I; Takouche B; Allen H (eds). Geneva.

ISO. (2006a). *ISO 4832: 2006, Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coliforms* – Colony – count technique, *3*.

ISO. (2015). *ISO 4833-1: 2013, Microbiology of the food chain – Horizontal method for the enumeration of microorganisms* – Part 1, *44*(0).

ISO (2004E). *ISO 6611: 2004 E Milk and Milk Products. Enumeration of colony – forming units.*

Jahreis, G., Hausmann, W. & Kiessling, G. (2001). Bioavailability of iodine from normal diets rich in dairy products-results of balance studies in women. *Exp Clin Endocrinol Diabetes 109(3):*163-167.

Jayatissa, R. & Gunathilaka, M. M. (2011). *Iodine Deficiency status of Children in Srilanka*, 2000 – 2001, Technical Report, Department of Nutrition, Medical Research Institute, Sri Lanka.

Kandhro, A. G., Razi, G. T. & Sirajuddhin, M. (2011). Comparison of urinary iodide Determination in Female thyroid patients by two techniques *Russian Journal of Electrochemistry 47*(12): 1355 – 1362.

Kazantzis, G. (2004). Cadmium, osteoporosis and calcium metabolism. *Biometals. 17*:493-98.

Kessler, J. H. (2004). The effect of supraphysiologic levels of iodine on patients with Cyclic Mystalgia. *Breast J. 10(4):* 328-338 [pubmedabstract].

Khan, A., Akhter, S. & Siddiqui, M. M. (2003). Continuous use of iodized salt may cause thyrotoxicosis in Plain Areas of North West Frontier Province (NWFP) of Poksitan. *Journal of Medical Sciences 3.*

Khan, A., Khan, A. M. M., & Akhtar, S. (2002). Thyroid Disorders, Etiology and prevalence. J. *Med. Sci 2(2):* 89 – 94.

Kharzan, M., Fereidoun, A. & Medi, H. (2003). A review on iodine determination methods in salt and biological samples. *Scimetr International Journal of Medical Sciences Scimetr*, 1(1): e14092 2003.

Knudsen, N., Bulow, I. & Jorgensen, T. (2001). Serum thyroglobulin a sensitive marker of thyroid abnormalities and iodine deficiency in epidemiologic studies. *J. Clin Endocrinol Metab 86*, 3599 – 3603.

Knudsen, N., Laurberg, P., Perrid, H., Biilow, I., Ovesen, L., & Jorgensten, T. (2002). "Risk factors by goiter and thyroid nodules". *Thyroid 12(10):* 879-88.

Kotwal, A., Priya, R., & Qadeer, I. (2007). Goiter and other deficiency disorders: A systematic Review of Epidemiological studies to deconstruct the complex web. *Archives of Medical Research 38*(1): 1 – 14.

Kramer, H. (2017). Kidney Disease and the Westernization and Industrialization of food, *American Journal of Kidney Disease*.

Kulkarni, S. P., Dhar, D. S., & Kulkarni, D. S. (2013). A rapid assessment method for the determination of iodate in table salt samples. *Journal of Analytical science and Technology 4*: 21.

Kwong, W. T., Friello, P. & Semba, R. D. (2004). Interactions between iron deficiency and lead poisoning: Idemiology and pathogenesis. *Sci Total Environ. 330*:21-37.

L'abbe, M. R. (2003). *Iodine, Properties and Determination*. Encyclopedia of Food Sciences and Nutrition, 20th ed.

Laurberg, P., Cerquueira, C., & Oversen, L. (2010). *Iodine intake as a determinant of shyroid disorders in populations. Best Pract Resclin endocrinol Metab 24*: 13.

Laurberg, P., Jorgenstein, T. & Perrild, H. (2006). The Danish investigation on iodine intake and thyroid disease, *Dan thyr 155:* 219 – 228.

Mandel, S. J. & Mandel, L. (2003). Radioactive iodine and the salivary glands. *Thyroid 13*:265-271.

Mansourin, A. R (2011). Metabolic Disorders of the Iodine Deficiency. *Pakistan Journal of Biological Sciences 14(7): 412-414*

Mao, I. F., Chen, M. L. & Ko, Y. C. (2001). Electrolyte loss in sweat and iodine deficiency in a hot environment. *Arch Environ Health 56*(3):271-277.

Maturin, L. & Peeler, J. T. (2001). Aerobic Plate Count. Bacteriological Analytical Manual. *Scientific Research, An Academic Publisher,1(1).*

Max, N. (2013). "How adding iodine to salt resulted in a decade's worth of IQ gains for the US. *Business insider*. (Retrieved 20/04/2017).

McDougall, I. R. & Cavalieri, R. R. (2000). In vivo radionuclide tests and imaging. In: Braverman LE, Utiger RD, (Eds). *Werner and Ingbar's the thyroid: A fundamental and clinical text*. Philadelphia, PA: Lippincott-Raven, 355-375.

Medani, M. H. A. M., Elnour, A. A. & Saeed, A. M. (2013). Excessive iodine intake, water chemicals and endemic goiter in a Sudanese Coastal Area. *Public Health Nutrition 16(9*): 1586 – 1592.

Melse, B. A. & Jaiswal, W. (2010). Iodine deficiency in pregnancy, infancy and childhood and its consequences for brain development. *Best Pract Res Clin Endocrinot Metab* 24**(**1**)**: 29-38 [pubmedabstract].

Millard, R. K., Saunders, M. & Palmer, A. M. (2001). Approximate distribution of dose among foetal organs radioiodine uptake via placenta transfer. *Phys Med Biol 46(11):*2773-2783.

Mobasseri, M., Roshanravan, N. & Alemdari M. N. (2014). Urinary and Milk Iodine status in Neonates and their Mothers during congenital Hypothyroidism Screening program in Eastern Azerbajan: A pilot study. *Iran J. Public Health 43* (10): 1380 – 1384.

Monass, A. (2014). Effect a salinity on the Fungal Occurrence in Al-Shega Area at Al-Qassim, Saudi Arabia. *Research Journal of Microbiology*, 9: 287 – 295.

Murray, C. W., Egan, S. K., Kim, H., Beru, N. & Bolger, P. M. (2008). US food and drugs administration's total diet study: Dietary intake of perchlorate and iodine: *J. Expo Sci. Environment Epidemiol*. 18*(6):* 571-580 [pubmedabstract].

Ni, Y. & Wang, Y. (2007). Application of chemometric methods to the simultaneous kinetic spectrophotometric determination of iodate and periodate based on consecutive reactions. *Microchem J. 86:* 216 – 226.

Ohashi, T., Yamaki, M., Pandav, C. S., Karmarkar, M. G. & Irie, M. (2000). Simple microplate method for determination of urinary iodine. *Clin Chem 46(4)*: 529 – 36 [PubMed].

Patrick, L. (2008). Iodine: Deficiency and Therapeutic Considerations. *Altern Med. Rev*. 13**(**2**)**: 116-127 [pubmedabstract].

Peeters, R., Fekete, C. & Goncalves, C. (2001). Regional physiological adaptation of the central nervous system deiodinases to iodine deficiency. *Am J Physiol Endocrinol Metab 281(1):*E54-E61.

Pereira, F. P., Ferreira, S., Lavill, I. & Bendicho, C. (2010). Determination of iodate in waters by cuvetteless, UV-Vis micro-spectrophotometry after liquid-phase microextraction. *Talanta 81*: 625 – 629.

Peterson, R.T., Link, B.A., Dowling, J.E., & Schreiber, S.L (2000). Classification of thyroid size by palpation and ultrasonography in field surveys. *Lancet, 355*:106–110.

Porth, C. M., Gaspard, K. J. & Noble, K. A. (2011). *Essential of pathophysiology: concepts of altered health states* (3rd ed.). Philadelphia. PA: Wolters Kluwer/Lippincott Williams & Wilkins.

Priya, R., Kotwal, A., & Qadeer. I. (2009). Toward an ecosocial epidemiological approach to goiter and other iodine deficiency disorders: a case study of iodine technocratic program for universal iodization of salt. *Int J Health Serv. 39(2):343-362.*

Shabani, A. M. H., Ellis, P. H. & McKelvie, I. D. (2011). Spectrophotometric determination of iodate in iodized salt by flow injection analysis. *Food chem 129*: 704 – 707.

Shaohua, W. (2000). *The final report of the expanded Iodine Dripping*. Project in Xingjian ygur autonomous region, 1997 – 1999, China.

Shen, D. H. Y., Kloos, R. T., Mazzaferri, E. L. & Jhiang, S. M. (2001). Sodium iodide symporter in health and disease. *Thyroid 11*(5):415-425.

Simon, S. L., Luckyanov, N. & Bouville, A. (2002). Transfer of ^{131}I into human breast milk and transfer coefficients for radiological dose assessments. *Health Phys 82(6):*796-806.

Soldin, P. O. (2002). Controversies in urinary iodine determinations. *Clin Biochem 35*(8): 575 – 579.

Soylak, M., Perker, D. S. & Turkoglu, O. (2008). Heavy metal contents of refined and unrefined table salts form Turkey, Egypt and Greece. *Environmental monitoring and assessment, 143* (1 – 3): 267 – 72.

Stephanie, L. L. (2014). *Iodine Deficiency: Background, Pathophysiology and Epidemiology,* Medscape Gritting, D. T. (Ed).

Stephanie, L. L., Elizabeth, N. P., & Sonia, A. K. (2015). Iodine deficiency. *Medscape*. (Retrieved 20/04/2017).

Tang, C. R., Su, Z., Lin, B. & Hung, H. (2010). *A novel method for iodate determination using cadmium sulfide quantum dots as fluorescent probes. Anal Chim Acta 678*: 203 – 207.

Taurog, A. (2000). Hormone synthesis: Thyroid iodine metabolism. In: Braverman LE, Utiger RD, (Eds). *Werner and Ingbar's the thyroid*: *A fundamental and clinical text*. (8th ed). Philadelphia, PA: Lippincott-Williams and Wilkins, 61-84.

Tuckermann, M. M. (1983). Analytical profiles of drug substances, 11: ed Klaus Florey. (ed) *Journal of Pharmacentical Sciences 72*(5).

Thomson/Micromedex. (2006). *Drug Information for the Health Care Professional*, Volume 1, Co. 2006.

Vandecasteele, C. M., Van Hees, M. & Hardeman F. (2000). The true absorption of ^{131}I, and its transfer to milk in cows given different stable iodine diets. *J Environ Radioact 47(3):*301-317.

Verheseen, R. H. & Schweitzer, C. M. (2008). Iodine deficiency, more than cretinism and goiter: *Med Hypotheses 71*(5): 645 – 8.

WHO & UNICEF (2004). *Report of the Technical Consultation to assess rapid test kits designed to monitor salt iodine content. New York, 28–29 September*. World Health Organisation and The United Nations Children's Fund.

WHO/UNICEF/ ICCIDD. (2001). *Assessment of iodine deficiency disorders and monitoring their elimination*. Geneva, World Health Organization, (WHO/ NHD/01.1).

Wiersinga, M. W. (2013). Smoking and thyroid. *Clinical Endocrinology 79*(12): 145 – 151.

Wolka, E., Shiferaw, S. & Biadgilign. S (2014). The effect of iodine-deficiency disorders on academic achievement of school children in southern Ethiopia. *Public Health Nutr 17*: 1120 – 4.

Wolka, E., Shiferaws, S. & Biadgilign, S. (2014). Epidemiological study of risk factors for goiter among primary school children in Southern Ethiopia. *Food Nutr Bull 35:* 20 – 27.

Zamazil, V. & Sterz, I. (2009). *Evaluation of iodine prophylaxix in the Czech Republic*, Comprehensive Handbook of iodine.

Zava, T. T. & Zava, T. D. (2011). Assessment of Japanese iodine intake based on seaweed consumption in Japan: A literature-based analysis. *Thyroid Research 4*: 14.

Zhao, J., Wang, P. & Shang, L. (2000). Endemic goiter associated with high iodine intake. *Am J Public Health 90(10)*:1633-1635.

Zimmermann, M. B. (2009). Iodine deficiency in pregnancy and the effects of maternal iodine supplementation on the offspring: A review. *Am J. Clin Nutrition*; *89*(2)-672S [pubmedabstract].

Zimmermann, M. B. (2009). Iodine deficiency. *Endoor Rev*. 30(4): 376-406 [pubmedabstract].

Zimmermann, M. B. (2003). Development of a dried whole-blood spot thyroglobulin assay and its evaluation as an indicator of thyroid status in goitrous children receiving iodized salt. *American Journal of Clinical Nutrition*, *77*:1453–1458.

Zimmermann, M. B. (2004). New reference values for thyroid volume by ultrasound in iodine-sufficient schoolchildren: a World Health Organization/Nutrition for Health and Development Iodine Deficiency Study Group Report. *American Journal of Clinical Nutrition*, *79*:231–237.

Zimmermann, M. B. (2006). Assessment of iodine status using dried blood spot thyroglobulin: development of reference material and establishment of an international reference range in iodine-sufficient children. *Journal of Clinical Endocrinology and Metabolism*, *91*:4881–4887.

Zimmermann, M. B., Jooste, P. L. & Pandav, C. S. (2008). Iodine-Deficiency Disorders. *Lancet* 372(9645): 1251-1262 [pubmedabstract].

Zimmermann, M. B. (2000). Thyroid ultrasound compared with World Health Organization 1996 and 1994 palpation criteria for determination of goitre prevalence in regions of mild and severe iodine deficiency. *European Journal of Endocrinology*, 143:727–31.

APPENDICES

APPENDIX A

Calculation of the Urinary Iodine Concentrations

(i) The Standard Calssical Titration Method

The formular was used to calculate the UIC of the urine samples

$$I_2 \text{ (mg/L)} = \frac{\text{titre value x 105.8 x 1000 x Df}}{\text{volume of water x } 10^6} \quad (10)$$

Where:

Df = dilution factor

The value obtained was converted to μg/L. The results are found in Appendix B.

(ii) Novel Method

The relation below was used to calculate the urinary iodine concentration in μg/L for the Novel method. The results are found in Appendices F – K.

$$\left(\frac{\left(Ms_2 0_3^{2-} Vs_2 0_3^{2-}(aq)\right) Df M_m{}^I x 10^6}{1000} \right) \mu g / L \quad (11)$$

$= 0.001 \times V_T \times Df \times 256$

$= V_T \times Df \times 0.256$

Where:

M = molarity of $Na_2S_2O_3$

V = volume of $Na_2S_2O_3$ in litres

Mm = molar mass of iodine

V_T = titre value

(iii) Sandell-Kolthoff Method

A standard curve was generated by Exel Programme to calculate the iodine concentration in the samples.

Absorbance vrs Concentration of Standard Iodine Solutions

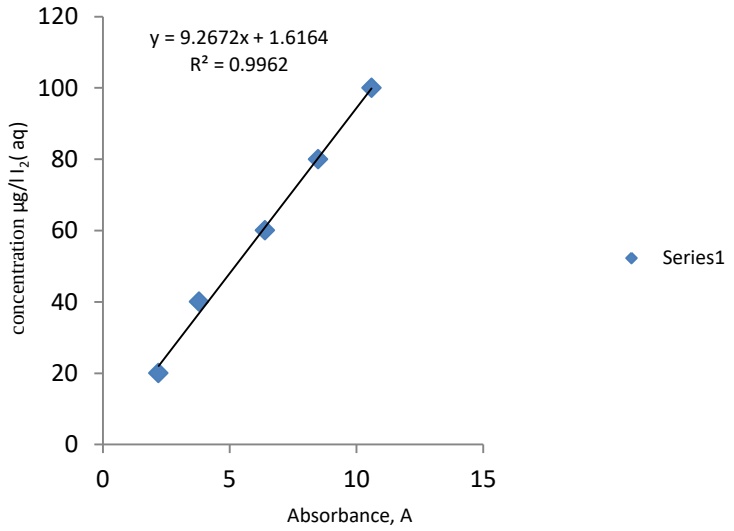
Absorbance (A)	**Concentration of standard solutions (µg/L)**
2.2001	20.104
3.8020	40.040
6.4100	60.120
8.5500	80.020
10.6111	100.140

The formular generated by the excel program is:

$$Y = 9.2672X + 1.6164 \quad (12)$$

Where Y is concentration of iodine and X is the absorbance

Calibration Curve of Concentration vrs Absorbance



Dilution factor = 25

The results obtained are found in Appendices C – F

APPENDIX B:

Urinary Iodine Concentration (UIC) for the 1048 urine samples determined by the Classical Titration Method

Sample	I_2 µg/L	Sample	I_2 µg/L	Sample	I_2 µg/L
1	85.86	22	95.46	43	63.6
2	56.18	23	36.04	44	74.25
3	53.06	24	42.46	45	32.86
4	33.92	25	40.28	46	23.32
5	44.52	26	21.23	47	22.26
6	53.01	27	40.28	48	25.44
7	46.64	28	78.44	49	42.47
8	40.28	29	44.52	50	32.86
9	33.92	30	39.87	51	26.56
10	48.76	31	27.56	52	29.68
11	55.12	32	42.44	53	86.92
12	42.41	33	63.62	54	190.8
13	31.82	34	74.25	55	42.44
14	63.65	35	43.46	56	38.16
15	42.46	36	44.52	57	46.64
16	64.66	37	36.04	58	25.44
17	55.12	38	33.92	60	33.92
18	44.52	39	47.77	61	21.20
19	75.26	40	74.25	62	22.26
		41	41.34	63	64.98
		42	75.47	64	45.58

APPENDIX B: Continued

65	67.84	89	47.74	113	24.98
66	43.46	90	37.11	114	56.18
67	72.08	91	28.62	115	15.94
68	64.66	92	63.62	116	8.48
69	32.86	93	74.24	117	43.42
70	63.61	94	30.74	118	33.92
71	84.80	95	85.86	119	43.46
72	22.26	96	86.92	120	75.26
73	40.28	97	39.22	121	77.38
74	87.98	98	33.92	122	45.58
75	100.73	99	68.93	123	55.12
76	75.26	100	79.52	124	85.86
77	96.46	101	36.04	125	32.86
78	97.52	102	24.38	126	23.32
79	31.84	103	45.58	127	85.72
80	43.46	104	44.52	128	74.23
81	45.58	105	63.64	129	24.38
82	42.44	106	46.64	130	20.14
83	39.22	107	18.02	131	40.28
84	31.82	108	96.46	132	84.81
85	58.35	109	26.52	133	18.02
86	43.46	110	9.54	134	28.62
87	51.94	111	42.43	135	22.26
88	23.32	112	29.68	136	28.62

APPENDIX B: Continued

137	40.28	161	22.26	186	64.66
138	75.26	162	61.48	187	27.06
139	53.34	163	40.28	188	39.22
140	30.74	164	26.51	189	65.72
141	84.82	165	58.31	190	43.46
142	33.92	167	43.46	191	15.94
143	43.46	168	69.96	192	27.56
144	81.62	169	76.32	193	106.44
145	64.66	170	92.22	194	46.64
146	85.86	171	74.22	195	76.32
147	43.46	172	31.86	196	41.62
148	67.84	173	31.82	197	37.44
149	116.61	174	39.22	198	44.72
150	95.42	175	33.92	199	54.08
151	47.72	176	27.56	200	43.68
152	31.83	177	45.58	201	33.28
153	24.38	178	27.56	202	39.52
154	38.16	179	20.14	203	43.68
155	27.56	180	22.26	204	75.92
156	40.28	181	11.66	205	29.12
157	27.56	182	54.11	206	31.23
158	38.16	183	44.52	207	43.08
159	20.14	184	54.06	208	60.32
160	31.84	185	47.72	209	54.08

APPENDIX B: Continued

210	45.76	234	47.84	258	89.44
211	73.84	235	46.80	259	43.68
212	28.05	236	66.56	260	70.96
213	55.12	237	58.24	261	92.56
214	39.52	238	43.68	262	93.61
215	44.72	239	52.46	263	58.24
216	46.83	240	35.36	264	81.12
217	36.45	241	49.92	265	29.12
218	40.56	242	58.24	266	36.43
219	42.64	243	75.92	267	60.32
220	64.48	244	61.36	268	38.48
221	76.96	245	72.82	269	49.92
222	45.76	246	45.76	270	74.88
223	60.32	247	55.12	271	37.44
224	65.52	248	52.58	272	43.68
225	66.56	249	63.44	273	29.12
226	83.25	250	28.08	274	106.56
227	75.92	251	64.48	275	72.82
228	29.12	252	58.24	276	35.36
229	47.84	253	46.56	277	37.44
230	44.72	254	37.44	278	43.68
231	74.88	255	53.04	279	64.48
232	43.68	256	95.68	280	87.36
233	39.52	257	45.76	281	49.92

APPENDIX B: Continued

282	54.08	306	29.12	330	47.84
283	58.24	307	27.04	331	87.36
284	56.16	308	43.68	332	31.22
285	76.96	309	64.48	333	79.04
286	30.16	310	34.32	334	56.16
287	74.88	311	55.12	335	33.28
288	45.76	312	37.44	336	70.72
289	85.28	313	45.76	337	41.63
290	34.32	314	47.84	338	47.84
281	29.12	315	39.52	339	43.68
292	64.48	316	43.68	340	54.08
293	70.72	317	31.22	341	56.16
294	82.44	318	47.84	342	49.92
295	74.88	319	85.28	343	37.44
296	28.08	320	66.56	344	45.76
297	74.88	321	47.84	345	64.48
298	34.32	322	37.44	346	65.52
299	41.62	323	58.24	347	43.68
300	45.76	324	29.12	348	84.24
301	48.88	325	33.28	349	29.12
302	65.52	326	64.48	350	31.22
303	47.84	327	65.52	351	37.44
304	58.24	328	70.72	352	31.22
305	35.36	329	93.6	353	24.96

APPENDIX B: Continued

354	29.12	378	81.12	402	68.64
355	21.84	379	79.04	403	38.48
356	28.08	380	72.82	404	54.08
357	37.44	381	33.28	405	59.28
358	64.48	382	76.96	406	72.82
359	39.52	383	66.56	407	89.44
360	54.08	384	91.52	408	47.84
361	45.76	385	68.64	409	74.88
362	52.24	386	48.88	410	35.36
363	41.62	387	60.32	411	94.64
364	47.84	388	64.48	412	47.84
365	41.62	389	87.36	413	19.76
366	85.28	390	72.82	414	59.28
367	56.16	391	58.24	415	27.04
368	76.96	392	78.35	416	29.12
369	53.04	393	70.72	417	70.96
370	74.88	394	83.22	418	58.24
371	75.96	395	47.84	399	43.68
372	25.26	396	85.28	420	39.52
373	68.64	397	68.64	421	45.76
374	87.36	398	64.48	422	43.68
375	89.44	399	73.84	423	39.52
376	41.62	400	29.12	424	41.60
377	47.84	401	45.76	425	4 7.84

APPENDIX B: Continued

426	58.24	450	58.24	474	62.42
427	76.96	451	38.34	475	87.3
428	64.48	452	41.6	476	43.68
429	70.72	453	79.04	477	65.52
430	62.43	454	84.24	478	30.16
431	64.48	455	85.28	479	74.88
432	60.32	456	47.84	480	45.76
433	68.64	457	56.16	481	54.08
434	62.43	458	29.12	482	64.48
435	64.48	459	58.24	483	35.36
436	30.16	460	74.88	484	58.24
437	85.28	461	43.68	485	52.40
438	71.76	462	42.64	486	39.52
439	32.24	463	38.48	487	65.52
440	36.52	464	94.64	488	54.08
441	56.16	465	62.4	489	22.88
442	54.08	466	63.44	490	29.12
443	52.24	467	30.16	491	72.80
444	64.48	468	48.88	492	44.72
445	47.84	469	88.4	493	49.92
446	74.88	470	71.76	494	63.44
447	62.42	471	54.08	495	37.44
448	45.76	472	62.42	496	29.12
449	29.12	473	41.62	497	63.44

APPENDIX B: Continued

498	32.24	522	50.96	546	59.28
499	27.04	523	63.44	547	33.04
500	41.64	524	35.36	548	34.32
501	73.84	525	50.96	549	78.04
502	73.84	526	66.56	540	29.12
503	34.32	527	48.88	551	67.62
504	43.68	528	58.24	552	42.64
505	34.48	539	31.20	553	28.08
506	84.24	530	38.48	554	62.42
507	57.20	531	21.84	555	88.42
508	43.68	532	42.64	556	70.72
509	66.56	533	86.32	557	64.48
510	38.48	534	82.16	558	44.72
511	56.16	535	67.60	559	43.68
512	74.88	536	33.28	560	37.44
513	98.80	537	37.44	561	75.92
514	50.98	538	74.88	562	95.68
515	31.22	539	47.84	563	47.84
516	38.48	540	89.44	564	41.60
517	34.32	541	73.84	565	68.64
518	53.04	542	49.92	566	76.96
519	65.52	543	88.42	567	48.88
520	42.64	544	31.20	568	36.41
521	43.68	545	36.40	569	58.24

Appendix B: Continued

570	67.62	594	42.64	618	86.32
571	70.72	595	49.92	619	53.04
572	75.92	596	73.84	620	73.84
573	54.08	597	43.64	621	44.72
574	59.28	598	66.56	622	28.08
575	29.12	599	66.56	623	64.48
576	71.76	600	56.16	624	56.16
577	94.64	601	27.04	625	35.36
578	66.56	602	60.32	626	73.84
579	86.32	603	32.24	627	58.24
580	62.43	604	44.72	628	34.32
581	48.88	605	37.44	629	65.52
582	36.41	606	38.48	630	45.76
583	43.68	607	65.52	631	62.40
584	52.04	608	43.68	632	52.24
585	32.24	609	36.44	633	37.44
586	37.44	610	40.56	634	76.96
587	57.20	611	84.24	635	92.56
588	46.82	612	59.28	636	88.4
589	21.84	613	26.5	637	74.88
590	47.84	614	42.64	638	55.12
591	38.48	615	24.96	639	36.44
592	76.96	616	57.24	640	36.44
593	34.32	617	52.40	641	44.72

Appendix B: Continued

642	30.16	666	29.12	690	64.48
643	62.40	667	33.28	691	35.36
644	52.24	668	33.28	692	60.32
645	36.40	669	26.46	693	74.88
646	47.84	670	42.64	694	46.84
647	38.48	671	96.72	695	39.52
648	22.88	672	54.08	696	84.24
649	27.04	673	23.92	697	31.20
650	19.76	674	17.68	698	62.40
651	20.8	675	44.72	699	60.32
652	17.68	676	27.04	700	42.64
653	32.24	677	37.44	701	37.44
654	95.68	678	27.04	702	75.92
655	38.48	679	37.44	703	53.04
656	26.24	680	34.32	704	32.24
657	20.82	681	62.4	705	23.92
658	96.72	682	44.72	706	46.84
659	59.28	683	30.16	707	64.48
660	86.32	684	24.96	708	54.08
661	23.92	685	65.52	709	75.92
662	29.12	686	27.04	710	83.24
663	53.04	687	46.8	711	43.68
664	44.72	688	33.28	712	65.52
665	36.43	689	47.84	713	38.48

Appendix B: Continued

714	23.92	738	57.20	762	64.48
715	37.44	739	63.44	763	58.24
716	89.44	740	34.32	764	40.26
717	32.24	741	54.08	765	40.56
718	40.56	742	34.32	766	34.32
719	36.40	743	43.68	767	63.44
720	28.08	744	64.48	768	34.48
721	37.44	745	48.88	769	44.72
722	72.86	746	29.12	770	73.84
723	44.72	747	24.96	771	44.72
724	54.08	748	58.24	772	30.16
725	65.52	749	67.60	773	23.92
726	24.96	750	74.92	774	65.52
727	27.04	751	92.56	775	35.36
728	34.32	752	64.48	776	36.42
729	60.32	753	73.84	777	27.04
730	64.48	754	37.52	778	43.68
731	78.24	755	23.92	779	26.45
732	43.68	756	53.04	780	36.4
733	66.56	757	37.44	781	32.24
734	56.16	758	33.28	782	65.52
735	64.48	759	58.24	783	53.04
736	72.8	760	29.12	784	55.28
737	43.68	761	43.68	785	64.48

Appendix B: Continued

786	53.04	810	53.04	834	74.88
787	35.36	811	47.84	835	35.36
788	78.00	812	43.68	836	29.12
789	64.48	813	43.68	837	76.96
790	27.04	814	69.68	838	65.52
791	43.68	815	64.48	839	52.00
792	65.52	816	33.28	840	71.76
793	42.64	817	43.68	841	43.68
794	32.24	818	54.08	842	63.44
795	30.16	819	44.72	843	38 .48
796	48.88	820	94.64	844	46.80
797	82.16	821	43.68	845	29.12
798	28.08	822	65.52	846	59.28
799	57.22	823	27.04	847	64.52
800	46.80	824	21.84	848	56.16
801	86.32	825	38.48	849	28.08
802	46.84	826	34.32	850	43.68
803	28.08	827	48.88	851	54.08
804	43.68	828	32.24	852	46.8
805	55.12	829	33.28	853	61.35
806	74.88	830	46.86	854	40.56
807	53.04	831	46.86	855	39.52
808	65.52	832	38.48	856	65.52
809	61.36	833	27.07	857	53.04

Appendix B: Continued

858	59.28	882	29.12	906	64.48
859	63.44	883	65.52	907	29.12
860	84.24	884	83.22	908	53.04
861	30.16	885	61.36	909	73.84
862	75.92	886	36.42	910	75.92
863	66.56	887	54.08	111	60.16
864	55.12	888	43.68	912	54.08
865	42.64	889	34.32	913	27.04
866	43.58	890	55.12	914	54.08
867	63.44	891	63.44	915	48.88
868	57.24	892	53.04	916	66.56
869	75.92	893	64.48	917	41.64
870	33.28	894	47.84	918	49.92
871	39.52	895	21.84	919	36.44
872	23.92	896	48.88	920	63.44
873	84.24	897	65.52	921	57.24
874	45.76	898	59.28	922	62.4
875	48.88	899	52.46	923	82.16
876	47.84	900	36.47	924	65.52
877	32.24	901	42.64	925	32.24
878	76.96	902	31.20	926	42.64
879	75.92	903	71.76	927	44.72
880	32.24	904	46.84	928	53.04
881	32.24	905	32.24	929	33.28

Appendix B: Continued

930	58.24	954	38.48	978	17.68
931	65.52	955	48.88	979	40.56
932	73.84	956	53.04	980	38.48
933	63.44	957	64.48	981	34.32
934	43.68	958	34.32	982	72.86
935	33.28	959	58.24	983	88.4
936	52.26	960	73.84	984	21.84
937	37.44	961	36.46	985	34.32
938	28.08	962	65.52	986	36.44
939	17.68	963	53.04	987	31.24
940	29.12	964	36.04	988	12.48
941	46.86	965	21.76	989	96.72
942	64.48	966	54.08	990	35.36
943	43.68	967	27.04	991	92.56
944	31.20	968	58.24	992	38.48
945	42.64	969	42.64	993	16.64
946	59.28	970	34.32	994	78.80
947	45.76	971	37.44	995	19.76
948	52.26	972	14.56	996	99.60
949	58.24	973	47.84	997	10.58
950	28.08	974	73.84	998	42.30
951	45.76	975	18.72	999	21.16
952	67.60	976	11.44	1000	31.74
953	42.64	977	32.24	1001	15.90

Appendix B: Continued

1002	56.18	1018	45.58	1034	10.6
1003	56.20	1019	53.00	1035	23.32
1004	10.60	1020	26.51	1036	63.61
1005	24.38	1021	33.92	1037	42.4
1006	24.38	1022	10.64	1038	21.2
1007	31.80	1023	0.00	1039	42.41
1008	31.81	1024	10.62	1040	0.00
1009	0.00	1025	0.00	1041	148.42
1010	22.26	1026	0.00	1042	31.80
1011	31.80	1027	21.24	1043	31.80
1012	21.22	1028	42.41	1044	31.80
1013	21.20	1029	31.82	1045	28.62
1014	10.61	1030	31.80	1046	148.4
1015	42.42	1031	31.80	1047	31.82
1016	0.00	1032	12.72	1048	183.38
1017	56.18	1033	0.00		

APPENDIX C

Absorbances and Concentrations of Staff Urinary Iodine by Sandell-Kolthoff

Sample	Absorbance, A	$I_2\ \mu g/l$	Sample	Absorbance, A	$I_2\ \mu g/l$	Sample	Absorbance, A	$I_2\ \mu g/l$
1	0.0002	40.46	22	0.0018	40.83	43	0.2012	87.02
2	0.0080	42.26	23	0.0006	40.55	44	0.0409	49.89
3	0.3000	109.91	24	0.0210	89.06	45	0.1013	63.88
4	0.0002	40.46	25	0.0018	40.83	46	0.1010	63.81
5	0.0090	42.49	26	0.0002	40.46	47	0.0811	226.09
6	0.0800	58.94	27	0.0411	49.93	48	1.3012	341.87
7	0.1800	82.11	28	.3810	128.68			
8	0.2000	86.75	29	0.2011	87.00			
9	0.0600	54.31	30	0.0411	49.93			
10	0.0600	54.31	31	0.2010	86.98			
11	0.880	244.29	32	0.0002	40.46			
12	0.0201	45.07	33	0.0002	40.46			
13	0.1000	63.58	34	0.0002	40.46			
14	0.0309	49.45	35	0.2013	75.38			
15	0.2401	96.04	36	0.4011	133.34			
16	0.0298	47.31	37	0.3812	128.73			
17	0.4609	147.19	38	0.3011	110.17			
18	0.2940	108.52	39	0.2211	91.63			
19	0.3009	110.12	40	0.0002	40.46			
20	0.2010	89.06	41	0.8011	226.09			
21	0.2960	108.99	42	0.1410	73.08			

APPENDIX D

Absorbances and Concentrations of Pupils Urinary Iodine by Sandell-Kolthoff

Sample	Absorbance, A	$I_2\ \mu g/l$	Sample	Absorbance, A	$I_2\ \mu g/l$	Sample	Absorbance, A	$I_2\ \mu g/l$
1	0.3012	120.00	22	0.3844	139.35	43	0.5441	176.48
2	0.2011	96.73	23	0.6404	198.82	44	0..2246	102.20
3	0.3114	122.38	24	0.2240	102.06	45	0.5942	188.22
4	0.3944	141.67	25	0.3870	139.95	46	0.2012	96.76
5	0.3827	138.95	26	0.3014	119.96	47	0.1427	83.15
6	0.3920	141.12	27	0.2421	106.27	48	0.1500	84.85
7	0.3026	120.33	28	0.2347	104.54	49	0.2103	98.87
8	0.2140	99.73	29	0.3867	139.81	50	0.2998	119.68
9	0.3010	119.96	30	0.3841	139.28	51	0.2048	97.59
10	0.3420	129.49	31	0.2014	96.80	52	0.2086	98.48
11	0.3940	141.58	32	0.4104	145.39	53	0.2210	101.36
12	0.2030	97.17	33	0.3814	138.65	54	0.6540	202.03
13	0.2340	104.38	34	0.3804	138.42	55	1.1021	306.22
14	0.4010	143.21	35	0.4026	143.18	56	0.3431	129.75
15	0.2210	101.36	36	0.3341	127.18	57	0.3041	120.05
16	0.6401	198.80	37	0.3140	127.66	58	0.4920	164.46
17			38	0.3110	122.98	59	0.2047	95.57
18	0.3641	134.63	39	0.2714	96.80	60	0.5014	166.55
19	0.3701	136.03	40	0.2241	102.08	61	0.2946	118.47
20	0.6420	199.24	41	0.6423	199.31	62	0.2010	96.71
21	0.3841	139.28	42	0.2241	102.08	63	0.2114	99.13

Appendix D: Continued

Sample	Absorbance, A	$I_2\ \mu g/l$	Sample	Absorbance, A	$I_2\ \mu g/l$	Sample	Absorbance, A	$I_2\ \mu g/l$
64	0.3110	122.28	85	0.3014	120.05	106	0.2750	113.91
65	0.1440	83.46	86	0.5780	184.36	107	0.1741	90.46
66	0.4947	164.99	87	0.5010	166.46	108	0.0003	50.05
67	0.3014	120.05	88	0.3514	131.68	109	0.4864	163.06
68	0.4010	143.21	89	0.2041	97.43	110	0.0003	50.05
69	0.4070	144.60	90	0.4914	164.86	111	0.0002	50.02
70	0.3742	136.98	91	0.3101	122.08	112	0.1743	90.50
71	0.4140	145.77	92	0.1941	95.11	113	0.0004	50.07
72	0.5947	188.24	93	0.2140	99.73	114	0.0472	60.95
73	0.3014	120.05	94	0.5442	167.50	115	0.1748	90.62
74	0.3211	124.13	95	0.4014	143.30	116	0.0003	50.05
75	0.5990	189.24	96	0.6448	199.89	117	0.3743	137.00
76	0.8010	236.21	97	0.3046	120.80	118	0.1540	85.78
77	0.6221	194.62	98	0.0462	60.72	119	0.0003	50.05
78	0.8141	239.26	99	0.1993	96.31	120	0.0033	50.74
79	0.8150	239.46	100	0.2018	96.90	121	0.2246	102.20
80	0.2998	119.68	101	0.4139	146.21	122	0.3471	130.68
81	0.2990	119.49	102	0.0574	63.32	123	0.1747	90.59
82	0.2994	119.59	103	0.0003	50.05	124	0.1529	85.53
83	0.2999	199.70	104	0.1543	85.85	125	0.3475	130.77
84	0.3040	120.66	105	0.1740	90.43	126	0.0004	50.07

Appendix D: Continued

Sample	Absorbance, A	$I_2\ \mu g/l$	Sample	Absorbance, A	$I_2\ \mu g/l$	Sample	Absorbance, A	$I_2\ \mu g/l$
127	0.0014	50.30	148	0.2420	106.24	169	0.2073	98.17
128	0.2475	107.52	149	0.1472	84.20	170	0.2042	97.45
129	0.3814	138.65	150	0.5876	186.59	171	0.2942	118.38
130	0.0015	50.33	151	0.3946	141.72	172	0.3010	119.96
131	0.0002	50.02	152	0.1406	82.67	172	0.0003	50.05
132	0.2015	96.83	153	0.0486	61.30	173	0.0013	50.28
133	0.3014	120.05	154	0.0004	50.07	174	0.0476	61.04
134	0.0002	50.02	155	0.0003	50.05	175	0.0003	50.05
135	0.0004	50.07	156	0.0013	50.28	176	0.0489	61.35
136	0.0003	50.02	157	0.0476	61.04	177	0.1476	84.29
137	0.0023	50.51	158	0.0003	50.04	178	0.0002	50.02
138	0.0474	61.00	159	0.0004	50.07	179	0.2440	106.71
139	0.2430	106.47	160	0.0004	50.07	180	0.2090	50.21
140	0.2040	97.41	161	0.0642	64.90	181	0.3884	140.28
141	0.0004	50.07	162	0.0003	50.05	182	0.1394	82.39
142	0.3887	140.35	163	0.2070	98.10	183	0.1476	84.29
143	0.1560	86.25	164	0.0408	59.46	184	0.2942	118.38
144	0.1346	81.27	165	0.0002	50.02	185	0.0456	60.58
145	0.3046	120.08	166	0.2942	118.38	186	0.2048	97.59
146	0.1474	84.25	167	0.0013	50.28	187	0.1747	90.59
147	0.4017	143.37	168	0.0464	60.76	188	0.0015	50.33

Appendix D: Continued

Sample	Absorbance, A	$I_2\ \mu g/l$
189	0.2042	97.45
190	0.1270	97.55
191	0.0473	60.97
192	0.0015	50.33
193	0.0010	50.21
194	0.5942	188.31
194	0.2080	98.34
195	0. 3464	130.51

APPENDIX E

Absorbance and Concetrations of Undergraduates Urinary Iodine by Sandell – Kolthoff

Sample	Absorbance, A	$I_2\ \mu g/l$	Sample	Absorbance, A	$I_2\ \mu g/l$	Sample	Absorbance, A	$I_2\ \mu g/l$
1	0.2743	65.20	32	0.4283	101.23	63	0.4752	112.10
2	0.2062	50.21	33	0.2186	52.44	64	0.3251	77.20
3	0.2511	60.00	34	0.2958	70.40	65	0.4077	96.42
4	0.3113	74.20	35	0.2962	70.50	66	0.5406	127.30
5	0.2812	67.30	36	0.4274	101.20	67	0.4938	116.42
6	0.2662	65.50	37	0.2778	66.20	68	0.3690	87.41
7	0.1253	53.02	38	0.2550	59.30	69	0.5023	118.41
8	0.2739	65.30	39	0.3384	80.30	70	0.2774	66.12
9	0.3801	90.10	40	0.3130	74.40	71	0.2821	07.20
10	0.1926	46.40	41	0.4223	99.80	72	0.3380	80.21
11	0.2107	50.60	42	0.3328	78.80	73	0.3044	72.40
12	0.3124	74.26	43	0.3363	79.80	74	0.3332	79.10
13	0.3818	90.40	44	0.3414	80.60	75	0.5098	120.14
14	0.3167	75.26	45	0.2911	69.30	76	0.3191	75.82
15	0.3217	76.42	46	0.2696	64.30	77	0.3294	78.20
16	0.4237	100.12	47	0.3515	83.34	78	0.2438	58.30
17	0.1922	46.30	48	0.4836	113.70	79	0.3732	88.40
18	0.3556	84.30	49	0.3410	80.90	80	0.4589	108.32
19	0.2756	65.70	50	0.3999	94.60	81	0.3685	87.30
20	0.3062	72.80	51	0.2963	70.30	82	0.2757	65.72
21	0.2773	66.10	52	0.2869	76.43	83	0.3088	73.42
22	0.2481	59.30	53	0.3218	68.32	84	0.4078	96.43
23	0.2627	62.70	54	0.3532	83.74	85	0.4421	104.40
24	0.2567	61.30	55	0.2016	48.50	86	0.3642	86.30
25	0.3922	92.80	56	0.3778	89.45	87	0.3818	90.40
26	0.4454	105.46	57	0.3174	75.42	88	0.3466	82.20
27	0.2939	70.10	58	0.3810	90.20	89	0.3681	87.15
28	0.3334	79.30	59	0.2517	60.14	90	0.4537	107.10
29	0.3663	86.80	60	0.2705	64.50	91	0.3373	80.08
30	0.4249	100.40	61	0.5638	132.70	92	0.4422	104.42
31	0.4464	104.70	62	0.3410	76.10	93	0.3677	87.10

Appendix E: Continued

Sample	Absorbance, A	$I_2\ \mu g/l$	Sample	Absorbance, A	$I_2\ \mu g/l$	Sample	Absorbance, A	$I_2\ \mu g/l$
94	0.5786	136.14	124	0.4582	108.14	154	0.3126	74.30
95	0.3393	80.50	125	0.3828	90.62	155	0.3216	76.40
96	0.3117	74.10	126	0.3528	75.80	156	0.3807	90.14
97	0.4025	95.21	127	0.2431	58.14	157	0.2601	62.10
98	0.4931	116.26	128	0.3732	88.40	158	0.2861	68.14
99	0.4634	109.37	129	0.2043	48.10	159	0.2400	57.41
100	0.5528	130.14	130	0.2356	56.40	160	0.2048	49.24
101	0.2443	58.41	131	0.3900	92.30	161	0.3552	84.20
102	0.4600	108.57	132	0.4077	96.40	162	0.3641	86.28
103	0.3332	39.10	133	0.4108	97.12	163	0.4799	113.20
104	0.3643	36.33	134	0.5265	124.04	164	0.2873	68.41
105	0.3332	79.10	135	0.3169	75.30	165	0.3685	87.30
106	0.3732	88.40	136	0.4855	114.50	166	0.3254	77.28
107	0.4330	102.30	137	0.3589	85.06	167	0.4197	99.20
108	0.3117	74.10	138	0.4937	116.40	168	0.3212	76.30
109	0.3732	88.40	139	0.4319	102.03	169	0.3376	80.12
110	0.3203	76.10	140	0.3552	84.20	170	0.3807	90.14
111	0.3203	58.30	141	0.4817	113.62	171	0.5105	120.32
112	0.2438	69.40	142	0.3460	82.06	172	0.3985	94.26
113	0.2915	74.10	143	0.3511	83.26	173	0.4425	104.50
114	0.3117	94.30	144	0.3162	75.14	174	0.3810	90.20
115	0.3972	68.40	145	0.503	83.06	175	0.4105	97.06
116	0.3633	86.10	146	0.4065	96.14	176	0.5106	120.34
117	0.3212	76.30	147	0.4240	100.20	177	0.3204	76.11
118	0.3548	84.10	148	0.2786	66.40	178	0.4581	108.12
119	0.3778	89.46	149	0.3818	90.40	179	0.5793	136.31
120	0.3219	76.46	150	0.4566	107.80	180	0.5111	120.44
121	0.3078	73.18	151	0.4679	110.41	181	0.3481	82.56
122	0.2434	58.20	152	0.4194	99.12	182	0.3204	76.12
123	0.3778	89.46	153	0.5541	130.44			

Appendix E: Continued

Sample	Absorbance, A	$I_2\ \mu g/l$	Sample	Absorbance, A	$I_2\ \mu g/l$	Sample	Absorbance, A	$I_2\ \mu g/l$
183	0.4378	103.42	213	0.4602	108.62	242	0.5627	132.44
184	0.4250	100.43	214	0.5611	132.08	243	0.5436	128.50
185	0.4384	103.55	215	0.3074	73.08	244	0.3139	74.60
186	0.2438	58.30	216	0.6451	151.60	245	0.2804	66.82
187	0.4889	115.30	217	0.4326	102.60	246	0.3745	88.70
188	0.3635	86.14	218	0.1960	47.20	247	0.3417	81.06
189	0.5885	138.46	219	0.1960	47.20	248	0.3812	90.26
190	0.4197	99.20	219	0.3942	93.28	249	0.4240	100.20
191	0.3634	86.12	220	0.2857	68.04	250	0.4872	114.90
192	0.4507	10.6.40	221	0.3573	84.70	251	0.4481	105.80
193	0.3720	88.06	222	0.4434	104.70	252	0.3741	88.60
194	0.4244	100.30	223	0.3948	93.40	253	0.4089	96.70
195	0.3976	94.06	224	0.3637	86.17	254	0.3287	78.04
196	0.3814	90.30	225	0.3546	84.07	255	0.4113	97.24
197	0.4172	98.62	226	0.3788	89.70	256	0.3203	76.08
198	0.4859	114.60	227	0.3711	87.89	257	0.3665	86.82
199	0.6560	154.14	228	0.3388	80.40	258	0.5068	119.46
200	0.3976	94.07	229	0.4025	95.20	259	0.6108	143.64
201	0.5577	131.28	230	0.3986	94.30	260	0.4730	111.60
202	0.4491	106.04	231	0.5840	125.78	261	0.3960	93.70
203	0.5248	123.64	232	0.4859	114.60	262	0.3660	86.72
204	0.4157	98.26	233	0.3836	90.80	263	0.2551	60.92
205	0.3922	92.80	234	0.4068	96.20	264	0.3879	91.80
206	0.5277	124.30	235	0.4670	110.20	265	0.5066	119.41
207	0.4319	102.04	236	0.4156	98.24	266	0.3879	91.80
208	0.3720	88.10	237	0.4434	104.70	267	0.3264	77.50
209	0.4022	95.14	238	0.3771	89.30	268	0.3106	73.84
210	0.3722	88.15	239	0.3836	90.80	269	0.6198	145.72
211	0.5826	137.08	240	0.5943	139.80	270	0.4507	106.41
212	0.6878	161.53	241	0.3158	75.04	271	0.4946	116.62

Appendix E: Continued

Sample	Absorbance, A	$I_2\ \mu g/l$	Sample	Absorbance, A	$I_2\ \mu g/l$	Sample	Absorbance, A	$I_2\ \mu g/l$
272	0.3394	80.52	302	0.5831	137.20	332	0.4554	107.50
273	0.3630	86.02	303	0.4307	101.76	333	0.6089	143.20
274	0.4980	117.41	304	0.4785	112.86	334	0.3397	80.60
275	0.4950	116.70	305	0.6902	162.09	335	0.4545	107.30
276	0.4439	104.42	306	0.6644	156.10	336	0.3340	72.40
277	0.4938	116.42	307	0.5849	137.62	337	0.4520	106.30
278	0.4694	110.75	308	0.3459	82.04	338	0.6128	144.09
279	0.5558	130.84	309	0.4163	98.04	339	0.5528	130.14
280	0.5751	134.62	310	0.4391	103.70	340	0.5681	133.70
281	0.4148	98.06	311	0.6495	152.65	341	0.3479	82.50
282	0.5296	124.76	312	0.5281	124.40	342	0.3603	85.40
283	0.3923	92.84	313	0.4543	107.24	343	0.5469	128.76
284	0.5560	130.90	314	0.6515	153.09	344	0.4674	110.30
285	0.4043	95.62	315	0.3900	92.30	345	0.6746	158.46
286	0.5959	130.90	316	0.4200	99.27	346	0.5860	137.86
287	0.5408	95.62	317	0.6993	164.42	347	0.5259	123.90
288	0.3956	140.17	318	0.6299	148.08	348	0.7164	168.19
289	0.5943	128.74	319	0.5797	136.41	349	0.3922	92.80
290	0.5109	93.60	320	0.4183	98.20	350	0.4464	105.40
291	0.4302	139.80	321	0.3548	84.10	351	0.4765	112.40
292	0.5946	120.40	322	0.4068	96.20	352	0.4865	114.73
293	0.5857	101.64	323	0.5714	134.47	353	0.3509	83.20
294	0.3676	139.86	324	0.6175	145.20	354	0.6032	141.86
295	0.3252	137.80	325	0.4291	101.38	355	0.4034	95.40
296	0.6675	77.24	326	0.4498	106.20	356	0.5062	119.30
297	0.4156	156.82	327	0.4111	97.20	357	0.3890	92.06
298	0.5210	98.24	328	0.5407	127.34	358	0.4141	87.90
299	0.6067	122.75	329	0.4150	98.10	359	0.5181	122.08
300	0.4228	99.92	330	0.4507	106.40	360	0.7248	170.14

Appendix E: continued

301	0.4593	107.48	331	0.6180	145.30	361	0.5533	130.27
362	0.5888	138.52	393	0.3724	88.20	425	0.6950	163.20
363	0.4197	99.20	394	0.2975	70.80	426	0.3767	89.20
364	0.4638	109.46	395	0.4240	100.20	427	0.3062	72.80
365	0.3900	92.30	396	0.3599	85.30	428	0.5203	122.60
366	0.6355	149.37	397	0.6154	144.70	429	0.4086	96.60
367	0.7718	181.06	398	0.3991	94.40	430	0.3351	79.52
368	0.4108	97.12	399	0.3735	88.45	431	0.5367	126.41
369	0.4460	105.32	400	0.3900	92.50	432	0.4434	104.72
370	0.5567	131.06	401	0.5324	125.41	433	0.5638	132.70
371	0.5627	132.45	402	0.4543	107.24	434	0.5130	120.90
372	0.4051	95.80	403	0.3995	94.50	435	0.4158	98.30
373	0.4975	117.30	405	0.4051	95.80	436	0.6412	150.72
374	0.5453	128.40	406	0.3668	86.90	437	0.5660	132.64
375	0.5294	124.70	407	0.4171	98.60	438	0.3080	72.52
376	0.5100	120.19	408	0.3079	73.20	439	0.5919	158.12
377	0.7036	168.20	409	0.3952	93.50	440	0.6068	142.70
378	0.5150	120.32	410	0.3832	90.72	441	0.6552	154.08
379	0.5208	122.70	411	0.3277	77.80	442	0.4606	108.70
380	0.3621	85.80	412	0.4885	115.20	443	0.4802	112.40
381	0.4980	117.40	413	0.4003	94.68	444	0.4846	114.30
382	0.6851	160.90	414	0.4025	95.20	445	0.4474	105.70
383	0.4681	110.46	415	0.4629	109.25	446	0.5341	125.80
384	0.5466	128.70	416	0.5296	124.76	447	0.3401	80.70
385	0.5332	125.60	417	0.5289	124.58	448	0.4148	98.07
386	0.3474	82.40	418	0.3406	80.80	449	0.4824	106.80
387	0.330	105.35	419	0.4434	104.70	450	0.3699	86.74
388	0.4008	94.80	420	0.2486	59.42	451	0.6762	157.82
389	0.3831	90.70	421	0.4639	109.48	452	0.3900	92.30
390	0.4200	99.28	422	0.4090	96.72	453	0.3668	86.90
391	0.3699	87.62	423	0.5552	130.70	454	0.3402	80.72
392	0.4068	96.20	424	0.5024	118.42	455	0.5021	118.36

Appendix E: Continued

Sample	Absorbance, A	$I_2\ \mu g/l$	Sample	Absorbance, A	$I_2\ \mu g/l$	Sample	Absorbance, A	$I_2\ \mu g/l$
456	0.3746	88.72	486	04434	103.72	516	0.5089	119.27
457	0.3825	90.24	487	0.4518	105.76	517	0.6022	141.62
458	0.5172	120.86	488	0.3976	92.72	518	0.4416	103.64
459	0.7865	183.70	489	0.2355	88.30	519	0.4117	96.43
460	0.4591	108.36	490	0.5618	130.46	520	0.4080	112.70
461	0.3642	86.30	491	0.4052	95.14	521	0.7720	180.14
462	0.4406	104.06	492	0.5017	117.30	522	0.4532	106.62
463	0.8448	197.12	493	0.3139	76.08	523	0.4619	108.12
464	0.5552	130.70	494	0.4567	106.72	524	0.4971	116.33
465	0.6326	148.62	495	0.4705	109.46	525	0.4629	108.42
466	0.4294	100.86	496	0.4288	100.46	526	0.4018	95.03
467	0.3805	89.16	497	0.4535	106.14	527	0.6211	146.03
468	0.4149	97.07	498	0.6169	144.16	528	0.5262	126.28
469	0.4675	109.36	499	0.4873	114.30	529	0.4388	103.64
470	0.4133	96.86	500	0.5004	117.14	530	0.4883	115.14
471	0.5164	120.74	501	0.5584	131.45	531	0.4373	102.46
472	0.4191	99.07	502	0.4583	108.16	532	0.3437	80.61
473	0.4536	106.14	503	0.5351	126.04	533	0.4853	113.40
474	0.4041	94.06	504	0.5128	120.84	534	0.5448	127.46
475	0.4980	116.42	505	0.4165	98.46	535	0.6138	143.60
476	0.4618	147.86	506	0.3913	92.60	536	0.6642	155.41
477	0.4663	109.16	507	0.6241	146.72	537	0.5142	120.63
478	0.3784	88.73	508	0.5037	118.74	538	0.6680	156.14
479	0.2546	99.70	509	0.4106	97.08	539	0.5068	119.46
480	0.5407	126.13	510	0.3912	92.57	540	0.6334	148.08
481	0.4897	144.72	511	0.4219	98.72	541	0.5314	124.58
482	0.4846	113.46	512	0.5415	127.52	542	0.5132	120.18
483	0.3711	87.04	513	0.4540	106.44	543	0.5391	126.17
484	4570	106.86	514	0.5681	144.62	544	0.5843	136.72
485	0.4472	104.72	515	0.5927	139.43	545	0.5935	139.60

Appendix E: Continued

Sample	Absorbance, A	$I_2\ \mu g/l$	Sample	Absorbance, A	$I_2\ \mu g/l$	Sample	Absorbance, A	$I_2\ \mu g/l$
546	0.6242	146.09	576	0.4433	104.16	606	0.7353	172.57
547	0.5045	118.30	577	0.4296	100.60	607	0.4625	109.16
548	0.4530	106.14	578	0.3846	90.12	608	0.4982	117.44
549	0.7983	186.34	579	0.6412	149.70	609	0.4390	103.68
550	0.5462	128.30	580	0.4861	114.08	610	0.6548	153.86
551	0.5067	118.70	581	0.3563	83.62	611	0.8335	195.40
552	0.3871	90.82	582	0.3461	81.04	612	0.6412	150.70
553	0.7243	109.03	583	0.5253	122.70	613	0.6687	157.09
554	0.7287	170.60	584	0.4060	95.24	614	0.7237	169.87
555	0.6533	153.07	585	0.4666	109.14	615	0.7016	165.79
556	0.8626	201.62	586	0.7998	186.70	616	0.6977	163.84
557	0.5729	134.60	587	0.7001	164.08	617	0.5540	130.41
558	0.5123	120.04	588	0.6691	156.70	618	0.5080	119.72
559	0.5396	127.08	589	0.6842	160.15	619	0.6323	148.64
560	0.3965	93.06	590	0.7399	173.14	620	0.5860	137.86
561	0.4901	114.60	591	0.5609	131.60	621	0.4625	109.14
562	0.4675	114.14	592	0.5572	130.46	622	0.5036	118.70
563	0.4326	101.338	593	0.7193	168.13	623	0.5032	118.62
564	0.5825	136.13	594	0.6533	153.06	624	0.4582	108.16
565	0.5410	126.73	595	0.3246	76.53	625	0.7702	180.70
566	0.4739	110.18	596	0.4764	111.30	626	0.4249	100.41
567	0.6000	140.28	597	0.7715	180.14	627	0.6584	154.70
568	0.5756	134.80	598	0.5302	124.53	628	0.2947	70.14
569	0.5211	122.08	599	0.6053	141.60	629	0.3717	88.04
570	0.5391	126.18	600	0.3919	110.14	630	0.5266	124.06
571	0.3878	91.04	601	0.6022	141.62	631	0.4508	106.42
572	0.6861	160.14	602	0.7764	182.14	632	0.4686	110.57
573	0.4452	104.63	603	0.4846	114.30	633	0.4636	109.40
574	0.4491	105.14	604	0.7222	169.52	634	0.4106	97.08
575	0.6717	156.90	605	0.5310	125.08	635	0.4880	115.09

Appendix E: Continued

Sample	Absorbance, A	$I_2\ \mu g/l$	Sample	Absorbance, A	$I_2\ \mu g/l$	Sample	Absorbance, A	$I_2\ \mu g/l$
636	0.5725	134.72	666	0.4638	109.46	696	0.6318	148.52
637	0.5344	125.87	667	0.6240	146.72	697	0.5655	133.09
638	0.4817	113.62	668	0.7028	165.02	698	0.5786	136.14
639	0.6538	153.63	669	0.9251	216.70	699	0.4950	116.70
640	0.4904	115.64	670	0.4391	103.71	700	0.4923	116.07
641	0.3832	90.72	671	0.4595	108.46	701	0.4950	116.72
642	0.6796	159.62	672	0.5795	136.36	702	0.6243	146.75
643	0.5137	121.06	673	0.4366	103.14	703	0.6075	142.86
644	0.4810	113.46	674	0.4743	135.14	704	0.5290	124.61
645	0.6514	153.06	675	0.5172	121.86	705	0.4337	102.46
646	0.4495	106.14	676	0.4583	108.16	706	0.4933	116.32
647	0.6994	164.25	677	0.4194	99.12	707	0.4582	108.14
648	0.4551	107.42	678	0.8946	209.62	708	0.7101	166.72
649	0.5197	122.46	679	0.4767	112.46	709	0.6042	142.10
650	0.4608	108.76	680	0.3765	89.16	710	0.4546	107.30
651	0.5783	136.07	681	0.5182	122.09	711	0.6732	158.14
652	0.6918	162.47	682	0.4866	114.76	712	0.5023	118.40
653	0.6417	150.82	683	0.7262	170.46	713	0.6945	163.08
654	0.4680	110.44	684	0.5672	133.48	714	0.7259	170.40
655	0.6247	146.86	685	0.4138	97.82	715	0.8020	188.09
656	0.6198	145.72	686	0.4742	111.86	716	0.4249	100.42
657	0.5813	136.78	687	0.4002	94.76	717	0.5334	126.10
658	0.5197	122.46	688	0.5671	133.46	718	0.4090	96.72
659	0.5928	139.44	689	0.8030	188.33	719	0.5290	124.64
660	0.6456	151.72	690	0.6366	149.62	720	0.5240	123.46
661	0.6727	158.02	691	0.4951	116.72	721	0.6911	162.30
662	0.5127	120.76	692	0.5356	126.14	722	0.6085	143.09
663	0.6757	158.72	693	0.3976	94.06	723	0.4981	117.43
664	0.6089	143.18	694	0.6241	146.72	724	0.5057	119.20
665	0.6932	162.78	695	0.6400	150.41	725	0.6816	160.09

Appendix E: Continued

Sample	Absorbance, A	$I_2\ \mu g/l$	Sample	Absorbance, A	$I_2\ \mu g/l$	Sample	Absorbance, A	$I_2\ \mu g/l$
726	0.5523	180.30	756	0.5020	118.34	786	0.4646	109.64
727	0.6386	150.09	757	0.6224	146.32	787	0.6275	147.51
728	0.7858	184.32	758	0.4410	104.32	788	0.8378	196.42
729	0.6128	144.10	759	0.4417	106.43	789	0.3114	74.02
730	0.4151	98.14	760	0.4495	106.12	790	0.3695	87.52
731	0.4638	109.46	761	0.5258	123.86	791	0.4085	96.62
732	0.5569	131.09	762	0.6515	153.09	792	0.3678	87.14
733	0.5733	134.92	763	0.3831	90.70	793	0.2340	56.00
734	0.5122	120.70	764	0.5811	136.72	794	0.8296	194.50
735	0.5702	134.20	765	0.7977	187.09	795	0.3694	87.50
736	0.6816	160.09	766	0.4025	96.20	796	0.8554	200.50
737	0.8455	198.20	767	0.7255	170.30	797	0.3259	77.40
738	0.5561	130.92	768	0.5286	124.52	798	0.2370	56.72
739	0.5109	120.40	769	0.4378	103.42	799	0.6581	154.60
740	0.4068	96.20	770	0.7002	164.42	800	0.1956	47.10
741	0.5380	162.71	771	0.5659	140.16			
742	0.4665	110.07	772	0.3888	92.03			
743	0.3638	86.20	773	0.5802	136.52			
744	0.3460	82.06	774	0.4597	108.50			
745	0.5010	118.10	775	0.4172	98.62			
746	0.5700	134.14	776	0.4151	98.13			
747	0.6128	144.09	777	0.2314	55.42			
748	0.4878	115.04	778	0.4581	108.12			
749	0.5160	121.60	779	0.7365	172.86			
750	0.4412	104.20	780	0.2126	51.04			
751	0.5368	126.42	781	0.1381	33.72			
752	0.5109	120.40	782	0.4158	98.30			
753	0.7676	130.02	783	0.2775	66.13			
754	0.5406	127.30	784	0.4767	112.46			
755	0.3746	88.71	785	0.4206	99.42			

APPENDIX F

Urinary Iodine Concentrations of the various analytical methods for Households

Sample	Classical Titration Method(µg/L)	Novel Method(µg/L)	Sandell – Kolthoff Method(µg/L)
1	59.60	81.14	90.46
2	10.58	31.74	40.28
3	42.30	84.64	93.78
4	21.16	52.90	64.09
5	31.74	52.90	70.28

APPENDIX G

Urine samples and average titre values for the staff population for the Novel method

Sample	Average titre/mL	Sample	Average titre/mL
1	3.50	25	2.30
2	7.30	26	5.40
3	11.1	27	12.10
4	3.00	28	8.00
5	3.82	29	5.00
6	6.00	30	8.40
7	7.53	31	3.20
8	8.00	32	2.00
9	5.40	33	3.55
10	5.33	34	8.22
11	6.04	35	13.10
12	4.40	36	12.30
13	6.32	37	1.00
14	5.14	38	9.00
15	9.00	39	4.12
16	5.22	40	21.20
17	12.42	41	7.22
18	9.00	42	8.00
19	1.22	43	5.10
20	8.30	44	28.30
21	9.24	45	29.00
22	4.00	46	6.40
23	4.12	47	33.00
24	4.54	48	2.30

APPENDIX H

Urinary Iodine Concentrations of the staff population by the Novel method

Sample	Average titre/mL	Sample	Average titre/mL
1	358.40	25	354.30
2	747.52	26	235.52
3	1136.64	27	552.96
4	307.20	28	1239.04
5	391.17	29	819.20
6	614.40	30	512.0
7	771.07	31	860.16
8	819.20	32	327.68
9	552.96	33	204.80
10	545.79	34	363.52
11	618.50	35	841.73
12	450.56	36	1341.44
13	647.17	37	1259.52
14	526.34	38	102.40
15	921.6	39	921.60
16	534.53	40	421.89
17	1271.81	41	2170.88
18	921.60	42	739.33
19	124.93	43	819.20
20	849.92	44	522.24
21	946.18	45	2897.92
22	409.60	46	2969.6
23	421.89	47	655.36
24	464.90	48	3379.20

APPENDIX I

Urine samples and average titre values of pupils by Novel method

Sample	Average titre/mL	Sample	Average titre/mL	Sample	Average titre/mL	Sample	Average titre/mL
1	9.00	27	8.26	53	16.30	79	10.00
2	7.08	28	12.00	54	24.00	80	10.46
3	9.42	29	9.44	55	10.10	81	10.00
4	11.00	30	8.42	56	9.76	82	10.60
5	9.82	31	9.10	57	8.89	83	11.22
6	9.92	32	9.38	58	8.00	84	9.00
7	9.74	33	11.20	59	15.00	85	13.14
8	8.20	34	13.14	60	9.64	86	14.18
9	9.44	35	8.44	61	7.00	87	9.88
10	9.52	36	9.74	62	7.40	88	8.00
11	7.48	37	9.08	63	9.90	89	11.02
12	10.66	38	7.76	64	6.00	90	10.00
13	8.00	39	8.90	65	14.21	91	6.62
14	10.00	40	16.11	66	9.10	92	8.77
15	8.63	41	8.76	67	11.09	93	15.24
16	14.60	42	14.18	68	12.30	94	9.22
17	9.32	43	8.12	69	9.66	95	16.26
18	9.04	44	15.98	70	12.00	96	9.20
19	13.56	45	6.99	71	14.22	97	4.90
20	9.40	46	6.09	72	7.00	98	6.24
21	12.00	47	6.22	73	9.48	99	7.76
22	14.12	48	7.40	74	14.54	90	10.00
23	7.70	49	9.33	75	19.10	91	6.62
24	9.08	50	7.48	76	16.18	92	8.77
25	8.40	51	8.02	77	21.44	93	15.24
26	8.12	52	7.27	78	21.86	94	9.22

Appendix I: Continued

Sample	Average titre/mL	Sample	Average titre/mL	Sample	Average titre/mL	Sample	Average titre/mL
95	16.26	111	6.50	137	5.58	163	4.66
96	9.20	112	3.78	138	8.64	164	3.00
97	4.90	113	4.50	139	7.00	165	8.72
98	6.24	114	7.32	140	4.26	166	4.00
99	7.76	115	4.12	141	9.00	167	5.22
100	9.46	116	10.00	142	6.40	168	8.00
101	5.68	117	6.00	143	5.74	169	8.42
102	3.60	118	4.20	144	9.30	170	8.86
103	5.86	119	5.78	145	6.64	171	9.00
104	6.00	120	8.20	146	11.34	172	4.28
105	8.11	121	9.22	147	7.42	173	4.88
106	6.28	122	5.69	148	7.80	174	5.66
107	3.10	123	6.32	149	15.24	175	4.20
108	13.12	124	9.33	150	10.00	176	5.00
109	3.78	125	4.26	151	6.62	177	6.42
110	2.44	126	4.00	152	4.30	178	3.34
111	6.50	127	8.44	153	3.21	179	8.00
112	3.78	128	9.21	154	4.86	180	3.20
113	4.50	129	3.50	155	3.88	181	10.42
114	7.32	130	2.88	156	4.62	182	6.14
115	4.12	131	6.42	157	3.12	183	6.40
116	10.00	132	9.00	158	4.77	184	5.64
117	6.00	133	2.86	159	3.66	185	6.90
118	4.20	134	3.22	160	5.78	186	7.60
119	5.78	135	3.46	161	4.00	187	3.90
110	2.44	136	3.22	162	7.86	188	7.52

Appendix I: Continued

Sample	Average titre/mL
189	6.88
190	5.00
191	3.62
192	3.00
193	13.00
194	8.40
195	9.20

APPENDIX J

Urinary Iodine Concentrations for Pupils population by Novel Method

Sample	$l_2\,\mu g/L$	Sample	$l_2\,\mu g/L$	Sample	$l_2\,\mu g/L$	Sample	$l_2\,\mu g/L$	Sample	$l_2\,\mu g/L$	Sample	$l_2\,\mu g/L$
1	457.20	27	419.61	53	828.04	79	508.00	98	316.92	124	473.96
2	359.66	28	609.60	54	1219.20	80	531.37	99	394.21	125	261.41
3	478.54	29	479.55	55	513.08	81	508.00	100	480.57	126	203.20
4	558.80	30	427.74	56	495.81	82	538.48	101	288.54	127	428.75
5	498.86	31	462.28	57	451.61	83	569.98	102	182.88	128	467.89
6	503.94	32	476.50	58	406.40	84	457.20	103	297.69	129	177.84
7	494.79	33	568.96	59	762.00	85	667.15	104	304.80	130	146.30
8	416.56	34	667.51	60	489.71	86	720.34	105	411.99	131	326.14
9	479.55	35	428.75	61	355.60	87	501.94	106	319.02	132	457.20
10	483.62	36	494.79	62	375.92	88	406.40	107	157.48	133	145.29
11	379.98	37	461.26	63	502.90	89	559.82	108	666.50	134	163.58
12	541.53	38	394.21	64	304.80	90	508.00	109	192.04	135	175.77
13	406.40	39	452.12	65	721.87	91	336.30	110	123.92	136	163.58
14	508.00	40	818.39	66	462.28	92	441.96	111	330.20	137	283.46
15	438.40	41	445.01	67	563.37	93	774.19	112	192.04	138	438.91
16	741.68	42	720.34	68	624.87	94	468.38	113	228.60	139	355.60
17	473.46	43	412.50	69	490.73	95	826.00	114	371.86	140	261.41
18	459.23	44	811.78	70	609.60	96	467.36	115	209.30	141	457.20
19	688.85	45	355.09	71	722.34	97	248.92	116	508.00	142	325.12
20	477.52	46	309.37	72	355.60	98	316.92	117	304.80	143	291.59
21	609.66	47	315.98	73	481.58	92	441.96	118	213.36	144	472.44
22	717.30	48	375.92	74	738.63	93	774.19	119	293.62	145	337.31
23	391.16	49	473.96	75	971.81	94	468.38	120	416.56	146	576.07
24	461.26	50	379.98	76	821.94	95	826.00	121	468.38	147	376.94
25	426.72	51	407.42	77	1,089.51	96	467.36	122	289.05	148	396.24
26	412.50	52	369.37	78	1,110.89	97	248.92	123	321.06	149	772.16

Appendix J: Continued

Sample	$l_2\,\mu g/L$	Sample	$l_2\,\mu g/L$
150	508.00	176	254.00
151	336.30	177	326.14
152	218.47	178	169.67
153	163.07	179	406.40
154	246.89	180	162.56
155	197.40	181	529.34
156	234.70	182	311.92
157	158.50	183	325.12
158	242.32	184	286.51
159	185.93	185	350.52
160	293.62	186	386.08
161	203.20	187	198.12
162	399.29	188	382.02
163	236.73	189	349.50
164	152.40	190	254.00
165	442.98	191	183.90
166	203.20	192	152.40
167	265.18	193	660.40
168	406.40	194	426.72
169	427.74	195	467.36
170	450.09		
171	457.20		
172	217.42		
173	247.42		
174	287.53		
175	213.36		

APPENDIX K

Titre value and urinary iodine concentrations for the Undergraduates Novel Method

Sample	Titre value, mL	$l_2\mu/L$	Sample	Titre value, mL	$l_2\mu/L$	Sample	Titre value, mL	$l_2\mu/L$
1	0.40	409.6	25	0.62	634.80	48	0.73	759.20
2	0.36	374.4	26	0.74	757.80	49	0.59	604.20
3	0.43	347.2	27	0.44	450.60	50	0.70	716.84
4	0.52	540.8	28	0.58	603.20	51	0.44	450.60
5	0.42	447.1	29	0.63	634.60	52	0.53	551.20
6	0.32	343.2	30	0.64	634.9	53	0.50	512.0
7	0.38	395.2	31	0.80	819.2	54	0.61	624.60
8	0.42	447.1	32	0.73	759.2	55	0.27	280.80
9	0.73	759.2	33	0.28	286.20	56	0.62	624.40
10	00.28	286.72	33	0.46	286.7	57	0.56	573.40
11	0.30	312.01	34	0.43	471.0	58	0.64	634.90
12	0.42	447.1	35	0.72	447.20	59	0.36	374.40
13	0.58	603.2	36	0.42	758.10	60	0.51	511.80
14	0.52	540.8	37	0.38	395.20	61	0.92	942.10
15	0.44	450.56	38	0.46	471.0	62	0.44	450.60
16	0.71	738.40	39	0.45	469.80	63	0.86	850.60
17	0.27	280.80	40	0.64	634.9	64	0.42	430.10
18	0.53	551.20	41	0.64	634.90	65	0.74	757.80
19	0.38	395.20	42	0.56	573.40	66	0.89	911.40
20	0.43	447.20	43	0.42	430.10	67	0.90	911.30
21	0.48	491.5	44	0.50	512.0	68	0.56	573.40
22	0.35	399.36	45	0.34	348.20	69	0.78	798.70
23	0.39	419.80	46	0.48	491.50	70	0.28	286.70
24	0.41	634.80	47	0.56	573.4	71	0.35	358.4

Appendix K: Continued

Sample	Titre value, mL	$l_2\mu/L$	Sample	Titre value, mL	$l_2\mu/L$	Sample	Titre value, mL	$l_2\mu/L$
72	0.58	603.20	96	0.28	286.70	120	0.38	395.50
73	0.37	378.90	97	0.62	634.90	121	0.42	430.10
74	0.48	491.50	98	0.68	696.30	122	0.30	316.60
75	0.72	759.10	99	0.86	880.60	123	0.46	430.10
76	0.36	374.40	100	0.72	759.10	124	0.82	839.70
77	0.42	430.10	101	0.27	280.80	125	0.64	655.40
78	0.28	286.70	102	0.72	759.10	126	0.46	430.10
79	0.64	655.40	103	0.33	337.90	127	0.36	374.40
80	0.70	716.80	104	0.40	409.60	128	0.56	573.40
81	0.34	348.20	105	0.44	450.60	129	0.28	286.70
82	0.36	374.40	106	0.47	481.20	130	0.32	343.20
83	0.42	430.10	107	0.63	645.10	131	0.62	634.90
84	0.62	634.90	108	0.46	430.10	132	0.63	640.10
85	0.84	860.20	109	0.56	573.40	133	0.68	696.30
86	0.48	491.50	110	0.34	348.20	134	0.90	921.60
87	0.52	540.8	111	0.28	286.70	135	0.46	430.10
88	0.56	573.40	112	0.26	266.20	136	0.84	860.20
89	**0.54**	**552.90**	113	0.42	430.10	137	**0.30**	**316.0**
90	0.74	757.80	114	0.62	634.90	138	0.76	778.20
91	0.29	296.90	115	0.33	337.90	139	0.54	552.90
92	0.72	759.10	116	0.53	542.70	140	0.32	343.20
93	0.44	450.60	117	0.36	374.40	141	0.68	696.30
94	0.82	839.70	118	0.44	450.60	142	0.40	409.60
95	0.33	337.90	119	0.46	430.10	143	0.46	430.10

Appendix K: Continued

Sample	Titre value, mL	$l_2\mu/L$	Sample	Titre value, mL	$l_2\mu/L$	Sample	Titre value, mL	$l_2\mu/L$
144	0.42	430.10	168	0.40	798.72	192	0.58	603.20
145	0.52	540.80	169	0.46	430.10	193	0.62	634.90
146	0.54	552.90	170	0.40	409.6	194	0.84	860.20
147	0.48	491.50	171	0.82	839.60	915	0.70	716.90
148	0.36	374.4	172	0.54	552.90	196	0.56	573.40
149	0.44	450.60	173	0.74	757.76	197	0.75	768.0
150	0.62	634.90	174	0.51	522.20	198	0.68	696.30
151	0.63	634.8	175	0.72	759.10	199	0.80	819.20
152	0.42	430.10	176	0.74	757.76	200	0.46	471.0
153	0.81	829.40	177	0.25	256.0	201	0.82	839.70
154	0.28	286.70	178	0.66	675.80	202	0.66	657.80
155	0.30	316.0	179	0.84	860.20	203	0.62	634.90
156	0.36	374.40	180	0.86	880.60	204	0.71	738.40
157	0.30	316.0	181	0.40	409.60	205	0.28	286.70
158	0.24	245.76	182	0.46	430.10	206	0.44	450.60
159	0.28	286.70	183	0.78	798.72	207	0.66	675.80
160	0.21	215.04	184	0.76	778.2	208	0.37	378.90
161	0.27	280.80	185	**0.70**	**716.80**	209	0.52	540.80
162	0.36	374.40	186	0.32	343.20	210	0.57	583.70
163	0.62	634.90	187	0.74	757.76	211	0.70	716.80
164	0.38	395.20	188	0.64	655.40	212	0.86	880.60
165	0.52	540.80	189	0.88	901.10	213	0.46	471.0
166	0.44	450.60	190	0.66	675.80	214	0.72	759.10
167	0.50	512.0	191	0.47	481.30	215	0.34	348.20

Appendix K: Continued

Sample	Titre value, mL	$l_2\mu/L$	Sample	Titre value, mL	$l_2\mu/L$	Sample	Titre value, mL	$l_2\mu/L$
216	0.91	286.70	240	0.62	634.90	264	0.56	573.40
217	0.46	471.0	241	0.29	297.0	265	0.72	737.30
218	0.19	194.60	242	0.82	839.70	266	0.42	430.10
219	0.57	583.70	243	0.69	706.60	267	0.41	419.80
220	0.26	286.70	244	0.31	317.40	268	0.37	378.90
221	0.28	286.70	245	0.38	395.20	269	0.91	931.80
222	0.74	575.80	246	0.54	552.90	270	0.60	614.40
223	0.56	573.40	247	0.52	532.50	271	0.61	624.60
224	0.42	430.10	248	0.50	512.0	272	0.29	297.0
225	0.38	389.10	249	0.62	634.90	273	0.47	481.30
226	0.44	450.60	250	0.46	471.0	274	0.85	870.40
227	0.42	430.10	251	0.72	759.10	275	0.69	706.60
228	0.38	395.20	252	0.60	614.40	276	0.52	540.80
229	0.40	409.60	523	0.44	450.60	277	0.60	614.40
230	0.46	471.0	254	0.28	286.70	278	0.40	409.60
231	0.56	573.40	255	0.56	573.40	279	0.60	614.40
232	0.74	757.80	256	0.37	378.90	280	0.84	860.20
233	0.62	634.90	257	0.40	409.60	281	0.42	430.10
234	0.68	696.30	258	0.76	778.2	282	0.63	645.10
235	0.60	614.40	259	0.81	829.40	283	0.29	296.90
236	0.62	634.9	260	0.82	839.70	284	0.72	737.30
237	0.58	603.20	261	0.46	471.0	285	0.44	540.80
238	0.66	675.80	262	0.54	552.90	286	0.52	634.90
239	0.60	614.40	263	0.28	286.70	287	0.62	348.20

Appendix K: Continued

Sample	Titre value, mL	$l_2\mu/L$	Sample	Titre value, mL	$l_2\mu/L$	Sample	Titre value, mL	$l_2\mu/L$
288.	0.34	573.40	312.	0.55	572.20	336.	0.21	218.40
289.	0.56	512.0	313.	0.42	436.80	337.	0.41	426.40
290.	0.50	395.20	314.	0.64	665.60	338.	0.83	863.20
291.	0.38	655.20	315.	0.38	395.20	339.	0.79	821.60
292.	0.64	540.80	316.	0.54	561.60	340.	0.65	676.0
293.	0.52	228.80	317.	0.72	737.20	341.	0.32	332.80
294.	0.22	291.20	318.	0.94	988.0	342.	0.36	374.40
295.	0.28	728.0	319.	0.50	509.60	343.	0.72	378.80
296.	0.70	447.20	320.	0.30	312.0	344.	0.46	478.40
297.	0.42	499.20	321.	0.37	384.80	345.	0.86	894.40
298.	0.48	634.40	322.	0.33	343.20	346.	0.71	738.40
299.	0.61	374.40	323.	0.51	530.40	347.	0.48	499.20
300.	0.36	291.20	324.	0.63	655.20	348.	0.85	884.0
301.	0.28	634.40	325.	0.41	426.40	349.	0.30	312.0
302.	0.60	322.40	326.	0.42	436.80	350.	0.35	364.0
303.	0.30	270.40	327.	0.49	509.60	351.	0.57	592.80
304.	0.26	416.0	328.	0.61	634.40	352.	0.51	530.40
305.	0.40	738.40	329.	**0.34**	**353.60**	353.	0.33	343.20
306.	0.72	738.40	330.	0.49	509.60	354.	0.75	780.0
307.	0.71	343.20	331.	0.67	665.60	355.	0.28	291.20
308.	0.32	436.80	332.	0.47	488.80	356.	0.65	676.0
309.	0.42	395.20	333.	0.56	582.40	357.	0.41	426.40
310.	0.38	842.40	334.	0.30	312.0	358.	0.27	280.80
311.	0.81	842.40	335.	0.37	384.80	359.	0.60	624.0

Appendix K: Continued

Sample	Titre value, mL	$l_2\mu/L$	Sample	Titre value, mL	$l_2\mu/L$	Sample	Titre value, mL	$l_2\mu/L$
360.	0.85	884.0	384.	0.83	863.20	408.	0.31	322.40
361.	0.68	707.20	385.	0.60	624.0	409.	0.43	447.20
362.	0.62	644.80	386.	0.47	488.80	410.	0.36	374.40
363.	0.43	447.20	387.	0.35	364.0	411.	0.37	384.80
364.	0.42	430.80	388.	0.42	436.8	412.	0.63	655.20
365.	0.36	374.40	389.	0.50	520.0	413.	0.42	436.80
366.	0.73	759.20	390.	0.31	322.40	414.	0.35	364.0
367.	0.92	956.80	391.	0.36	374.4	415.	0.39	405.60
368.	0.46	478.40	392.	0.55	572.0	416.	0.81	842.40
369.	0.40	616.0	393.	0.45	468.0	417.	0.57	592.80
370.	0.66	686.40	394.	0.21	218.40	418.	0.25	260.0
371.	0.74	769.60	395.	0.46	478.40	419.	0.41	426.40
372.	0.47	488.80	396.	0.37	384.80	420.	0.24	249.60
373.	0.35	364.0	397.	0.74	769.60	421.	0.55	572.0
374.	0.56	582.40	398.	0.33	343.20	422.	0.50	520.0
375.	0.65	676.0	399.	0.41	426.40	423.	0.83	863.20
376.	0.68	707.20	400.	0.48	499.20	424.	0.51	530.40
377.	**0.73**	**759.20**	401.	0.71	738.40	425.	**0.71**	**738.40**
378.	0.52	540.8	402.	0.42	436.80	426.	0.43	447.20
379.	0.57	592.80	403.	0.64	665.60	427.	0.27	280.80
380.	0.28	291.20	404.	0.64	665.60	428.	0.62	644.80
381.	0.69	717.60	405.	0.54	561.60	429.	0.54	561.60
382.	0.91	946.40	406.	0.26	270.40	430.	0.34	353.60
383.	0.64	665.6	407.	0.58	603.2	431.	0.71	738.40

Appendix D: Continued

Sample	Titre value, mL	$l_2\mu/L$	Sample	Titre value, mL	$l_2\mu/L$	Sample	Titre value, mL	$l_2\mu/L$
432.	0.56	582.40	456.	0.20	208.0	480.		447.20
433.	0.33	343.20	457.	0.17	176.80	481.	0.26	270.40
434.	0.63	655.20	458.	0.31	322.40	482.	0.36	374.40
435.	0.44	457.60	459.	0.92	956.80	483.	0.26	270.40
436.	0.60	624.0	460.	0.37	384.80	484.	0.36	374.40
437.	0.50	520.0	461.	0.25	260.0	485.	0.33	343.20
438.	0.36	374.40	462.	0.20	208.0	486.	0.60	624.0
439.	0.74	769.60	463.	0.93	967.20	487.	0.43	447.20
440.	0.89	925.60	464.	0.57	592.80	488.	0.29	301.60
441.	0.85	884.0	465.	0.83	863.20	489.	0.24	249.60
442.	0.72	748.80	466.	0.25	239.20	490.	0.63	656.20
443.	0.53	551.20	467.	0.28	291.20	491.	0.26	270.40
444.	0.35	364.0	468.	0.51	530.40	492.	0.45	468.0
445.	0.35	364.0	469.	0.43	447.20	493.	0.32	332.0
446.	0.43	447.20	470.	0.35	364.0	494.	0.45	478.7
447.	0.29	301.60	471.	0.28	291.20	495.	0.62	644.80
448.	0.60	624.0	472.	0.32	332.80	496.	0.34	353.60
449.	0.50	520.0	473.	**0.32**	**332.80**	497.	0.58	603.20
450.	0.35	364.0	474.	0.25	260.0	498.	0.72	748.80
451.	0.46	478.40	475.	0.41	426.40	499.	0.45	468.0
452.	0.37	384.80	476.	0.93	967.20	500.	0.38	395.20
453.	0.22	228.80	477.	0.52	540.80	501.	0.81	842.40
454.	0.26	270.40	478.	0.23	239.20	502.	0.30	312.0
455.	0.19	197.60	479.	0.17	176.80	503.	0.60	624.0

Appendix K: Continued

Sample	Titre value, mL	$l_2\mu/L$	Sample	Titre value, mL	$l_2\mu/L$	Sample	Titre value, mL	$l_2\mu/L$
504.	0.58	603.20	528.	0.43	477.20	552.	0.24	249.60
505.	0.41	426.40	529.	0.52	540.80	553.	0.56	582.40
506.	0.36	374.40	530.	0.65	655.20	554.	0.65	676.0
507.	0.73	759.20	531.	0.24	249.60	555.	0.73	759.20
508.	0.51	530.40	532.	0.26	270.40	556.	0.89	925.60
509.	0.31	322.40	533.	0.33	343.20	557.	0.62	644.8
510.	0.23	239.20	534.	0.58	603.20	558.	0.71	738.40
511.	0.45	468.0	535.	0.62	644.80	559.	0.38	395.20
512.	0.62	644.80	536.	0.75	780.0	560.	0.23	239.20
513.	0.52	540.80	537.	0.42	436.80	561.	0.51	530.40
514.	0.73	759.20	538.	0.64	665.60	562.	0.36	374.40
515.	0.80	832.0	539.	0.54	561.60	563.	0.32	332.80
516.	0.42	436.80	540.	0.62	644.80	564.	0.56	582.40
517.	0.63	655.20	541.	0.70	728.0	565.	0.28	290.20
518.	0.37	384.80	542.	0.42	436.80	566.	0.42	436.80
519.	0.23	239.20	543.	0.55	572.0	567.	0.62	644.80
520.	0.36	374.40	544.	0.61	634.40	568.	0.56	532.40
521.	**0.86**	**894.40**	545.	0.33	343.20	569.	**0.25**	**260.0**
522.	0.31	322.40	546.	0.52	540.80	570.	0.39	405.60
523.	0.39	405.60	547.	0.33	343.20	571.	0.33	343.20
524.	0.35	364.0	548.	0.42	436.80	572.	0.61	634.40
525.	0.27	280.80	549.	0.62	644.80	573.	0.37	384.80
526.	0.36	374.40	550.	0.47	488.80	574.	0.43	447.20
527.	0.70	728.0	551.	0.28	291.20	575.	0.71	738.40

Appendix K: Continued

Sample	Titre value, mL	$l_2\mu/L$	Sample	Titre value, mL	$l_2\mu/L$	Sample	Titre value, mL	$l_2\mu/L$
576.	0.43	447.20	600.	0.29	301.60	624.	0.43	447.20
577.	0.29	301.60	601.	0.47	488.80	625.	0.91	946.40
578.	0.23	239.20	602.	0.79	821.60	626.	0.42	436.80
579.	0.63	655.20	603.	0.27	280.80	627.	0.63	655.20
580.	0.34	353.60	604.	0.55	572.00	628.	0.26	270.40
581.	0.35	364.00	605.	0.45	468.00	629.	0.21	218.40
582.	0.26	270.40	606.	0.83	863.20	630.	0.37	384.80
583.	0.42	436.80	607.	0.45	488.80	631.	0.33	343.20
584.	0.25	260.0	608.	0.27	280.80	632.	0.47	488.80
585.	0.35	264.00	609.	0.42	436.80	633.	0.31	322.40
586.	0.31	322.4	610.	0.53	551.20	634.	0.32	332.80
587.	0.63	655.20	611.	0.72	748.80	635.	0.45	468.00
588.	0.51	530.40	612.	0.51	530.40	636.	0.45	468.00
589.	0.57	592.80	613.	0.63	655.20	637.	0.37	384.80
590.	0.62	644.80	614.	0.52	613.60	638.	0.26	270.40
591.	0.51	530.40	615.	0.51	530.40	639.	0.72	748.80
592.	0.34	353.60	616.	0.46	478.40	640.	0.34	555.60
593.	0.75	780.00	617.	**0.42**	**435.80**	641.	0.28	291.20
594.	0.62	644.80	618.	0.42	436.80	642.	0.74	709.60
595.	0.27	270.40	619.	0.67	696.80	643.	0.63	655.20
596.	0.42	436.80	620.	0.62	644.80	644.	0.50	520.00
597.	0.63	655.20	621.	0.32	332.80	645.	0.69	717.60
598.	0.41	426.40	622.	0.42	436.80	646.	0.42	436.80
599.	0.31	322.40	623.	0.82	540.80	647.	0.61	634.40

Appendix K: Continued

Sample	Titre value, mL	$l_2\mu/L$	Sample	Titre value, mL	$l_2\mu/L$	Sample	Titre value, mL	$l_2\mu/L$
648.	0.37	384.80	672.	0.61	634.40	696.	0.61	634.40
649.	0.45	468.00	673.	0.55	572.00	697.	0.51	530.40
650.	0.28	291.20	674.	0.73	759.20	698.	0.62	644.80
651.	0.57	592.80	675.	0.32	332.80	699.	0.46	478.40
652.	0.63	655.20	676.	0.38	395.20	700.	0.21	218.40
653.	0.54	561.60	677.	0.23	239.20	701.	0.47	488.8
654.	0.27	280.80	678.	0.81	842.40	702.	0.63	655.20
655.	0.42	436.80	679.	0.44	457.60	703.	0.57	592.80
656.	0.52	540.80	680.	0.47	488.80	704.	0.50	520.00
657.	0.45	468.00	681.	0.46	478.40	705.	0.35	364.00
658.	0.59	613.50	682.	0.31	322.40	706.	0.41	426.40
659.	0.39	405.60	683.	0.74	769.60	707.	0.30	312.00
660.	0.38	389.12	684.	0.73	759.20	708.	0.69	717.60
661.	0.63	655.20	685.	0.31	322.40	709.	0.45	468.00
662.	0.51	530.40	686.	0.31	322.40	710.	0.31	322.40
663.	0.57	592.80	687.	0.28	291.20	711.	0.62	644.80
664.	0.61	634.40	688.	0.63	655.20	712.	0.28	291.20
665.	**0.88**	**842.40**	689.	0.80	832.00	713.	**0.51**	**530.40**
666.	0.29	301.60	690.	0.59	613.60	714.	0.71	738.40
667.	0.73	759.20	691.	0.35	364.00	715.	0.73	759.20
668.	0.64	665.60	692.	0.52	540.80	716.	0.29	301.60
669.	0.53	530.00	693.	0.42	436.80	717.	0.52	540.80
670.	0.41	426.40	694.	0.33	343.20	718.	0.26	270.40
671.	0.42	436.80	695.	0.53	551.20	719.	0.52	540.80

Appendix K: Continued

Sample	Titre value, mL	$l_2\mu/L$	Sample	Titre value, mL	$l_2\mu/L$	Sample	Titre value, mL	$l_2\mu/L$
720.	0.47	488.80	744.	0.17	176.80	768.	0.51	530.40
721.	0.64	665.60	745.	0.28	291.20	769.	0.35	364.00
722.	0.40	416.00	746.	0.45	468.00	770.	0.69	717.60
723.	0.48	499.20	747.	0.62	644.80	771.	0.52	540.80
724.	0.35	364.00	748.	0.42	436.80	772.	0.26	270.40
725.	0.61	634.40	749.	0.30	312.00	773.	0.56	582.40
726.	0.55	572.00	750.	0.41	426.40	774.	0.41	426.40
727.	0.60	624.00	751.	0.57	552.80	775.	0.33	343.20
728.	0.79	821.60	752.	0.44	457.60	776.	0.36	374.40
729.	0.63	655.20	753.	0.50	520.00	777.	0.14	145.6
730.	0.31	322.40	754.	0.56	582.40	778.	0.46	478.40
731.	0.41	426.40	755.	0.27	280.80	779.	0.71	738.40
732.	0.43	447.20	756.	0.44	457.60	780.	0.18	187.20
733.	0.51	530.40	757.	0.65	676.00	781.	0.11	114.40
734.	0.32	332.80	758.	0.41	426.40	782.	0.31	332.10
735.	0.56	532.40	759.	0.37	384.80	783.	0.17	176.80
736.	0.63	655.20	760.	0.47	448.80	784.	0.39	405.00
737.	0.71	738.40	761.	**0.51**	**530.40**	785.	0.37	384.60
738.	0.61	634.40	762.	0.62	644.80	786.	0.33	343.20
739.	0.42	436.80	763.	0.33	343.20	787.	0.70	728.00
740.	0.32	332.80	764.	0.56	582.40	788.	0.85	884.00
741.	0.50	520.00	765.	0.71	738.40	789.	0.21	218.40
742.	0.36	374.40	766.	0.35	364.00	790.	0.33	343.20
743.	0.27	280.80	767.	0.63	655.20	791.	0.35	364.00

Appendix K: Continued

Sample	Titre value, mL	$l_2\mu/L$
768.	0.30	312.00
769.	0.12	124.80
770.	0.93	967.20
771.	0.34	353.60
772.	0.89	925.60
773.	0.37	384.80
774.	0.16	166.40
775.	0.75	780.0
776.	0.29	197.60

APPENDIX L

Urinary Iodine Concentrations for households by Novel Method

Sample	Titre value, mL	I_2 µg/L
1	7.66	389.13
2	3.00	152.40
3	8.00	406.40
4	5.20	264.16
5	5.00	264.16

APPENDIX M

Conversion chart for salt iodine

Titre value/mL	I_2/ppm	Titre value/mL	I_2/ ppm	Titre value/mL	I_2/ppm	Titre value/mL	I_2/ppm
0.0	0	2.5	26.5	5.0	52.9	7.5	79.4
0.1	1.1	2.6	27.5	5.1	54.0	7.6	80.4
0.2	2.1	2.7	28.6	5.2	55.0	7.7	81.5
0.3	3.2	2.8	29.6	5.3	56.1	7.8	82.5
0.4	4.2	2.9	30.7	5.4	57.1	7.9	83.6
0.5	5.3	3.0	31.7	5.5	58.2	8.0	84.6
0.6	6.3	3.1	32.8	5.6	59.2	8.1	85.7
0.7	7.4	3.2	33.9	5.7	60.3	8.2	86.8
0.8	8.5	3.3	34.9	5.8	61.4	8.3	87.8
0.9	9.5	3.4	36.0	5.9	62.4	8.4	88.9
1.0	10.6	3.5	37.0	6.0	63.5	8.5	89.9
1.1	11.6	3.6	38.1	6.1	64.5	8.6	91.0
1.2	12.7	3.7	39.1	6.2	65.6	8.7	92.0
1.3	13.8	3.8	40.2	6.3	66.7	8.8	93.1
1.4	14.8	3.9	41.3	6.4	67.7	8.9	94.2
1.5	15.9	4.0	42.3	6.5	68.8	9.0	95.2
1.6	16.9	4.1	43.4	6.6	69.8	9.1	96.3
1.7	18.0	4.2	44.4	6.7	70.9	9.2	97.3
1.8	19.0	4.3	45.5	6.8	71.9	9.3	98.4
1.9	20.1	4.4	46.6	6.9	73.0	9.4	99.5
2.0	21.2	4.5	47.6	7.0	74.1	9.5	100.5
2.1	22.2	4.6	48.7	7.1	75.1	9.6	101.6
2.2	23.3	4.7	49.7	7.2	76.2	9.7	102.6
2.3	24.3	4.8	50.8	7.3	77.2	9.8	103.7
2.4	25.4	4.9	51.9	7.4	78.3	9.9	104.7

Source: WHO A Guide for Programme Managers, (2017)

APPENDIX N

Calculation of magnesium content in salt

Calculating the molarity of EDTA

Mass of $CaCO3_{(s)}$ = 0 .5010g

Molar mass CaCO3 = 100.0893g

Moles CaCO3 = 0.5010g / 100.0893g per mole = 0.005005 moles

This moles is contained in 500 mL solution, therefore 1000 mL will contain (1000/500) x 0.005005, ie, 0.010011 moles.

20.56 mL of EDTA titre volume will contain (25/100) x 0.010011 moles

Therefore 1000 mL of EDTA will contain 0.0122 moles and hence 0.0122M.

Thus the molarity of the EDTA is 0.0122M.

Sample calculation

Titration of salt solution with 0.0122 M EDTA solution

Table of titre values

	Titre1	Titre 2	Titre 3
Final volume/mL	12.72	25.40	38.10
Initial volume/mL	0.00	12.72	25.40
Difference/mL	12.72	12.72	12.70

The average titre of EDTA used = (12.72+12.72+12.70) / 3= 12.72 mL

The reaction equation: $Mg^{2+} + H_2Y^{2-} = MgY^{2-} + 2H^+$

Calculation:

1000 mls of EDTA solution contained 0.0122 moles

Therefore 12.72 mls will contain $1.55x10^{-4}$ moles

From the reaction equation 1mole Mg^{2+} = 1 mole EDTA, thus moles Mg^{2+} = moles EDTA= $1.55x10^{-4}$ moles.This is contained in 25 mL of salt solution, therefore 500 mL will contain $3.104\ x10^{-3}$ moles

Molar mass of Mg^{2+} = 24.312 g/mol, hence prcent mass of Mg^{2+} in 500 mL of solution is 0.25 %.

The rest of the results are found in Table 69.

APPENDIX O

Salt moisture content in salt

Sample calculation for week 1

Action	Values
Mass of dish/g	132.0000
Mass of dish+salt/g	142.0000
Mass of salt/g	10.0000
Mass of dish + salt after 1 week/g	142.0010
Absorbed moisture/g	0.0010
% moisture	0.05

Hence the moisture absorbed by the salt after week 1 is 0.05 %. The results for the remaining weeks are presented in Table 69.

APPENDIX P

Parameters for Determining Elements in Salts by Atomic Absorption Spectrophotometer

Element	comment	Slit width/ nm	Lamp mode	Lamp current/ mA	Burner height/ mm	Burner lateral/ pulse	Flame type	Fuel gas/L/ min	Support gas/L/ min	Wavelength/ nm	Burner angle/degree
Cd	Flame continuos	0.7	BCG-D2	8	7.0	0	Air-C_2H_2	1.8	15.0	228.8	0
Fe	Flame continuos	0.2	BCG-D2	12	9.0	0	Air-C_2H_2	2.2	15.0	248.3	0
Pb	Flame continuos	0.7	BGC-D2	10	7.0	0	Air-C_2H_2	2.0	15.0	283.3	0
Zn	Flame continuos	0.7	BGC-D2	8	7.0	0	Air-C_2H_2	2.0	15.0	213.9	0
Al	Flame continuos	2	BGC-D2	3	4.0	0	Nitrous oxide-C_2H_2		13.2	309.3	0

APPENDIX Q

Standard Solutions and their Concentrations by Atomic Absorption Spectrophotometer

Standard solution	Cd, ppm	Zn, ppm	Pb, ppm	Fe, ppm	Al, ppm
1	0.0568	0.0511	0.0640	0.0753	
2	0.0966	0.1117	0.5250	0.4625	
3	0.1905	0.1818	1.0326	1.0545	
4	0.5084	0.5060	1.9779	2.0564	
5	0.9977	0.9994	5.0005	2.9513	
blank	-0.0012	-0.0422	0.2624	-0.0630	

APPENDIX R

Educational Background of Patients

Educational Background	Frequency	Percent
Illiterate	10	14.0
Basic/Primary	14	20.0
Secondary	20	29.0
Tertiary	17	24.0
Middle School Leaving Certificate	9	13.0

APPENDIX S

Storage of Salt

Storage	Frequency	Percent
No idea	10	14.0
In a Container open	12	17.0
Salt dispenser	3	4.0
Close contaner	29	41.0
In the package	1	1.0
Container	13	19.0
Anyhow	1	1.0
Do not cook	1	1.0

APPENDIX T

Reasons for Preferring Iodised Salt

Reason	Frequency	Percent
	33	65.0
Because of its health benefits	1	14.0
Because of its benefits	1	4.0
Because it prevents goitre	1	10.0
Eliminates goitre	1	2.0
It contains iodine	1	2.0
It is medicinal	1	2.0
Protects against illness	1	2.0

APPENDIX U

Knowledge about Iodine Deficiency Disorders (Goitre)

Reason	Frequency	Percent
No idea	25	36.0
Caused by demons/evil/spirits	5	7.0
A disease	15	21.0
A growth in the throat/ expansion of thyroid gland	17	24.0
A swelling due to lack of iodine	7	10.0
Adams apple	1	1.0

APPENDIX V

Frequency of Use of Salt

Response	Frequency	Percent
No idea	9	13.0
Often/always	51	73.0
Daily	6	9.0
Not often	4	6.0

APPENDIX W

Preference for Type of Salt

Preference	Frequency	Percent
Non iodated	4	6.0
Any/Both/All	15	21.0
Iodated	51	73.0

APPENDIX X

Reasons for Preferring Non-iodised Salt

Reasons	Frequency	Percent
Use to it/familiar with it	1	50.0
Occasionally eat Annapurna	1	25.0
Not available in my town	1	25.0

APPENDIX Y

Preference for Brand of Salt

	Response	Frequency	Percent
	No idea	8	21.0
	Annapurna	22	57.0
	U2	4	10.0
	Annapurna/U2	1	3.0
	Any	3	8.0
	Total	**38**	**97.0**
Missing	System	1	3.0

APPENDIX Z:

Duration of Use of Salt

Duration	Frequency	Percent
No idea	9	13.0
Less than 10 yrs	54	77.0
More than 10 yrs	5	7.0
Long time/ since childhood	2	3.0

APPENDIX Z-1

Number of Goitrogenous Foods Eaten

Variables (n = 299)	No Goitre	Goitre	Inferential Statistics
9	100	0	Cramér's V = 0.3575
10	100	0	
11	75	25	
12	55	45	
13	87	13	
14	84	16	
15	85	15	
16	85	15	
17	60	40	
	33	67	

APPENDIX Z-2

Frequency of Use of Iodised Salt

Often/always	72	28	Cramér's V = 0.1855
Daily	89	11	
Not often	64	36	

APPENDIX Z-3

Age of Respondent

10-19 yrs	96	4	Cramér's V = 0.5945
20-29 yrs	88	12	
30-39 yrs	48	52	
40-49 yrs	24	76	
50-59 yrs	29	71	
60-69 yrs	60	40	
70-79 yrs	0	100	

APPENDIX Z-4

Educational Attainment

No formal education	0	100	Cramér's V = 0.6414
Basic/Primary	46	54	
MSLC	10	90	
Secondary	38	63	
Tertiary	91	9	

APPENDIX Z-5

Number of Goitrogenous Foods (Ref: Less)

Goitre Status	OR	SE	P-Value	[95% Conf.	Interval]
More	1.12103	0.053367	**0.016**	1.021163	1.230662

APPENDIX Z-6

Age (ref: 10-19 yrs) and Goitre Status

Age (ref: 10-19 yrs)	OR	SE	P-Value	95% Conf.	Interval]
20-29	1.548549	0.36681	0.065	0.9734149	2.463497
30-39	4.996672	1.650351	0.000	2.615395	9.546062
40-49	11.99723	5.206503	0.000	5.124844	28.08546
50-59	9.850065	5.371159	0.000	3.382875	28.68087
60-69	3.616977	2.295192	0.043	1.042813	12.54541
70-79	3622829	3708226	0.000	487283.5	2.69E+07

APPENDIX Z-7

Gender (Ref: Females) and Goitre Status

Gender (Ref: Females)	OR	SE	P-Value	[95% Conf.	Interval]
Male	0.265992	0.04546	**0.000**	0.190279	3.72E-01

APPENDIX Z-8

Educational Attainment (Ref: No formal education) and Goitre Status

Educational Attainment (Ref: No formal education)	OR	SE	P-Value	[95% Conf.	Interval]
Basic/Primary	1.77E-07	7.28E-08	**0.000**	7.87E-08	3.96E-07
MSLC	1.04E-06	1.08E-06	**0.000**	1.35E-07	7.97E-06
Secondary	2.32E-07	9.97E-08	**0.000**	1.00E-07	5.39E-07
Tertiary	4.56E-08	1.50E-08	**0.000**	2.39E-08	8.68E-08

APPENDIX Z-9

Knowledge on Iodised Salt (Ref: No) and Goitre Status

Knowledge on Iodised Salt (Ref: No)	OR	SE	P-Value	[95% Conf.	Interval]
Yes	0.155243	0.110856	**0.009**	0.0382987	0.629271

APPENDIX Z-10

Frequency of Use of Iodised Salt (Ref: Often) and Goitre Status

Frequency of use of Iodised Salt (Ref:Often)	OR	SE	P-Value	[95% Conf.	Interval]
Most Often	0.562126	0.11082	**0.003**	0.3819659	0.827261
Not often	1.23267	0.292931	0.379	0.7736918	1.963928

APPENDIX Z11

Negative log-log Multivariate Regression Model Predicting Diagnosed Goitre

Goitre Status	OR	SE		P-Value		[95% Conf.		Interval]	OR	SE		P-Value		[95% Conf.		Interval]	OR
	(Goitrogenous foods +Biosocial)					(Model 1+ Knowledge)										(Model 2+Behavioural)	
Number of goitrogenous foods (Ref: Less)																	
More	1.00897	0.066492	0.892	0.886713	1.148082	1.02558	0.077446	0.738	0.884488	1.189178	1.026081	0.077945	0.735	1.190809			
Model 1:goitrogenous foods + biosocial vs goiter																	
Age (ref:10-19 yrs)																	
20-29			2.063704	0.647414	**0.021**	1.115869	3.816646	1.593134	0.481666	0.123	0.880849	2.881398	1.586922	0.481825	0.128	2.877394	0.875209
30-39			4.378614	1.642165	**0.000**	2.099424	9.132153	3.411264	1.329767	**0.002**	1.588924	7.323652	3.530168	1.399064	**0.001**	7.6761	1.623492
40-49			9.41314	4.488126	**0.000**	3.697319	23.96526	5.563053	2.788509	**0.001**	2.082783	14.85875	5.683772	2.863458	**0.001**	15.25696	2.117411
50-59			7.831539	4.598502	**0.000**	2.477655	24.75446	4.022391	2.459644	**0.023**	1.213346	13.33472	4.010086	2.480513	**0.025**	13.4797	1.192964
60-69			1.782216	1.19965	0.391	0.476428	6.666892	9.30E-08	2.18E-08	**0.000**	5.87E-08	1.47E-07	1.31E-07	3.62E-08	**0.000**	2.25E-07	7.66E-08
70-79			2.00E+09	2.13E+09	**0.000**	2.47E+08	1.61E+10	2.08E-06	.	.	.	.	1.85E-06	2.00E-06	**0.000**	1.54E-05	2.21E-07
Gender (Ref: Females)																	
Male			0.314595	0.070063	**0.000**	0.20332	0.486769	0.367109	0.096693	**0.000**	0.219077	0.615167	0.383562	0.104996	**0.000**	0.655908	0.2243
Model 2:education and knowledge vs goitre																	
Educational Attainment (Ref: No formal education)																	
Basic/Primary	2.43E-15	.		.		.		.	7.63E-15	3.36E-15	**0.000**	1.81E-14	3.22E-15				
MSLC	6.65E-15	.		.		.		.	2.10E-14	8.32E-15	**0.000**	4.56E-14	9.66E-15				
Secondary	1.54E-15	.		.		.		.	4.73E-15	.	**.**	.					

APPENDIX Z11: Contineud

Tertiary	3.50E-09	.	.	.	.	7.91E-09	4.25E-09	**0.000**	2.27E-08	2.76E-09	
Knowledge on Iodated Salt (Ref: No)											
Yes	0.29017	0.140852	0.011	0.112065	0.75134	0.201928	0.143183	**0.024**	0.810525	0.050307	
Model 3:behaviourial vs goiter											
Frequency of use of Iodised Salt (Ref:Often)											
Most Often									1.036229	0.25891	0.887
Not often									0.58121	0.266621	0.237

APPENDIX Z-12

Do You Know Consuming Iodised Salt Cures Iodine Deficiency Disorders?

Response	Frequency	Percent
No	10	83.0
Yes	2	17.0

APPENDIX Z-13

Do You Consume Millet?

Response		Frequency	Percent
	No	5	13.0
	Yes	33	85.0
	Total	38	97.0
Missing	System	1	3.0

APPENDIX Z-14

College of Agriculture and Natural Sciences
School of Physical Sciences
Department of Chemistry
University of Cape Coast
Cape Coast

8th June, 2016

The Headmaster
Calvary Hillcrest Schools
Cape Coast

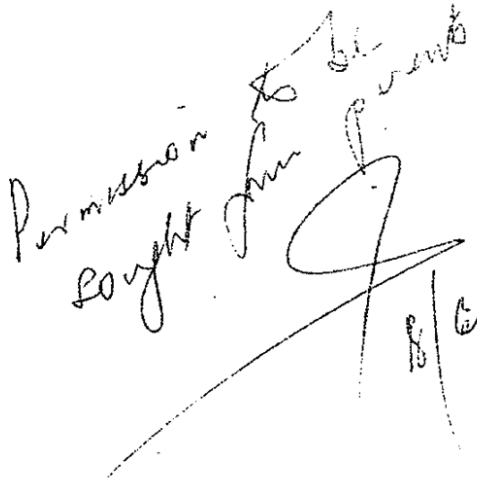
Dear Sir,

APPLICATION TO COLLECT URINE SAMPLES

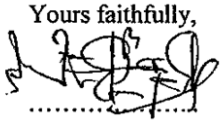
I wish to collect human urine samples from pupils of your institution. The preferred age bracket is 5-10years.

This exercise is to determine their iodine status for a PhD research at the above institution. The research aims at the impact of consumption of iodated salt in eliminating Iodine Deficiency Disorders (IDDs) such as cretinism (deficient intellectual capabilities in children) in Ghana.

Counting on your usual kind consideration.



Yours faithfully,



Benjamin Bartels

APPENDIX Z-15

College of Agriculture and Natural Sciences
School of Physical Sciences
Department Of Chemistry
University of Cape Coast
Cape Coast

19th August, 2016

Dear Sir,

APPLICATION FOR HUMAN URINE SAMPLES

I wish to apply for human urine samples from 2016/2017 level 100 students of University of Cape Coast from the hospital.

I am a second year PhD students from the above institution. My research work requires the use of human urine samples from 2016/2017 levels 100 students of University of Cape Coast, for which am ethical clearances has been obtained from UCCIRB (Photocopy attached).

Counting on your usual kind consideration.

The Administrator
U.C.C. Hospital
Cape Coast

Yours faithfully,

Benjamin Bartels
(PS/CHD/15/0001)
0271259998
0263967191

APPENDIX Z-16

QUESTIONNAIRE

UNIVERSITY OF CAPE COAST

COLLEGE OF AGRICULTURE AND NATURAL SCIENCES

SCHOOL OF PHYSICAL SCIENCES

DEPARTMENT OF CHEMISTRY

CAPE COAST

PHD CHEMISTRY PROGRAMME

1. GENDER: .....
2. AGE: .....
3. CLASS: .....
4. ACADEMIC PERFORMANCE: .....
5. i) DO YOU CONSUME EDIBLE SALT? Y N

   ii) IS THE SALT IODATED? Y N
6. i) DO YOU CONSUME CABBAGE? Y N

   ii) IS IT COOKED OR RAW? C R

APPENDIX Z-17

QUESTIONNAIRE FOR ADULTS

PHD CHEMISTRY PROGRAMME
UNIVERSITY OF CAPE COAST
COLLEGE OF AGRICULTURE AND NATURAL SCIENCES
SCHOOL OF PHYSICAL SCIENCES
CHEMISTRY DEPARTMENT
CAPE COAST

PROFILE

Sex: ……..

Age:……

Height:----------

Weight:----------

Blood Pressure:----------

Educational Background:……

KNOWLEDGE ABOUT IODISED SALT

Do you know iodised salt?

What are the benefits to human beings?

How often do you use it?

How do you store it?

Between iodised and non-iodised salts, which do you prefer? Please explain.

……

……

What brand do you like most?

Why do you like that brand?

How long have you been using it?

KNOWLEDGE ABOUT IODINE DEFICIENCY DISORDERS

What do you know about goiter (cretinism)?

How does it affect one's work/health?

What is the economic importance of IDDs?

Do you know that consuming iodised salt prevents these diseases?

IODINE STATUS

Do you know that it is possible to check iodine status?

Have you checked your iodine status?

Are you iodine deficient or sufficient?

THE LEGISLATIVE ACT

Do you know about the law that mandates all edible salt to be iodised in the Ghana?

Which government agency is to supervise the iodisation of edible salt?

Which government agency is to enforce that only iodised salt is sold on the market?

KNOWLEDGE ABOUT GOITROGENS

A. i) Do you drink or use tap water in cooking?
   ii) Do you drink or use borehole water in cooking?
   iii) Do you drink or use river water in cooking?
   iv) Do you drink or use rain water in cooking?

B. i) Do you eat cabbage, cooked or raw?
   ii) How often do you consume it?

C. i) Do you eat soy food (soyabean product)?
   ii) How often do you consume it?

D. i) Do you eat millet or millet product?
   ii) How often do you consume it?

E. i) Do you smoke cigarette?
   ii) How often do you smoke it?

F. i) Do you eat peas or any of it products?
   ii) How often do you consume it?

Do you know that these items contain substances that prevent uptake of iodine by the thyroid gland in the human body?

APPENDIX Z-18

UNIVERSITY OF CAPE COAST
INSTITUTIONAL REVIEW BOARD SECRETARIAT
TEL: 03321-33172/3 / 0207355653/ 0244207814
C/O Directorate of Research, Innovation and Consultancy
E-MAIL: irb@ucc.edu.gh
OUR REF: UCC/IRB/A/14
YOUR REF:
24TH JUNE, 2016

Mr. Benjamin Bartels
Department of Chemistry
University of Cape Coast

Dear Mr. Bartels,

ETHICAL CLEARANCE –ID NO: (UCCIRB/CANS/2016/01)

The University of Cape Coast Institutional Review Board (UCCIRB) has granted **Provisional Approval** for implementation of your research protocol titled: **": Levels of iodine in urine and iodated salt samples and iodine deficiency disorders in Ghana."**

This approval requires that you submit periodic review of the protocol to the Board and a final full review to the UCCIRB on completion of the research. The UCCIRB may observe or cause to be observed procedures and records of the research during and after implementation.

Please note that any modification of the project must be submitted to the UCCIRB for review and approval before its implementation.

You are also required to report all serious adverse events related to this study to the UCCIRB within seven days verbally and fourteen days in writing.

Always quote the protocol identification number in all future correspondence with us in relation to this protocol.

Yours faithfully,

(Samuel Asiedu Owusu)
ADMINISTRATOR

cc: The Chairman, UCCIRB

ADMINISTRATOR
INSTITUTIONAL REVIEW BOARD
UNIVERSITY OF CAPE COAST
Date: 26-06-16