Iodine status of lactating mothers and infants aged 0 to 6 months in Vhembe and Mopani district of the Limpopo Province, South Africa.

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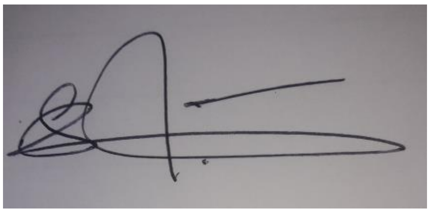
DECLARATION

I, **Seemole Cedrick Hlako (11618373),** hereby declare that the dissertation for the degree of Master's in Public Nutrition at the University of Venda, hereby submitted by me, has not been submitted previously for a degree at this or any other university, and that it is my own work in design and in execution, and that all reference materials contained herein has been duly acknowledged.

Signature: 

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DEDICATION

This work is dedicated to God Almighty for giving me support, strength, wisdom and the tenacity to complete this project successfully, and to my mother for her unconditional love and support.

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ABSTRACT

Introduction: Iodine is an essential nutrient required by humans for the synthesis of thyroid hormones, which are vital for normal growth and development.

Objective: The primary aim of the study was to describe the iodine status of lactating mothers and infants aged from 0 to 6 months in the Vhembe and Mopani Districts.

Methods: A cross-sectional study conducted on 246 infant-mother pair, from the Mopani and the Vhembe Districts. Data was gathered using a questionnaire. Breastmilk, mother urine, infant urine, household salt and drinking water were collected to be analysed for iodine content.

Results: The median of breastmilk iodine concentration level amongst lactating mothers in the Vhembe District was 101.4 µg/l (IQR 62.9 – 175.1 µg/l) and 154.4 µg/l (IQR 92.6 – 211.8 µg/L) in Mopani. The median UIC of mothers in Vhembe was 98.5 (IQR 57.66 – 153.93), whereas in the Mopani District the median UIC of mothers was 126.08 µg/l (IQR 69.89 – 206.71 µg/L). The median UIC of infants in Vhembe was 220 (IQR 106.67 – 418.43 µg/l) and in the Mopani District was 321.94 µg/l (IQR 167.96 – 482.66 µg/l).

Conclusion: The BMIC in the study signifies iodine sufficiency in both the Vhembe and the Mopani Districts. The results of this study suggest that the BMIC be included in studies assessing iodine status in lactating mothers since the UIC only reflects iodine that was consumed recently. The UIC may under estimate the maternal iodine status if it is not complemented by the BMIC data.

Keywords: Iodine, Goiter, Lactation, Breast milk iodine concentration, Urinary iodine concentration.

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DEFINATIONS OF OPERATIONAL TERMS

Breast milk – Milk produced by a woman s breast after child birth as food for her baby. Human milk contains a balance of nutrients that closely matches infant’s s requirements for brain development, growth and a healthy immune system.

Iodine - One of the trace elements essential to human health present in uneven and mostly insufficient quantities in the environment around the globe. It is an important substrate for the synthesis of thyroid hormones.

Iodine Deficiency – Lack or shortage of dietary iodine intake that results in impaired hormone synthesis leading to a series of functional and developmental abnormalities.

Goitrogens – Dietary substances that interfere with thyroid metabolism, which aggravate the effects of iodine deficiency.

LIST OF ABBREVIATIONS

BMIC Breast Milk Iodine concentrations

CDC Center for Disease Control cm

Centimeters DIT Diiodothyrosine

HH Household

HIV/AIDS Human Immune Virus/ Acquired Immune Deficiency Syndrome

ICCIDD International Council for Control of Iodine Deficiency Disorders

ID Iodine Deficiency

IDD Iodine Deficiency Disorders

IQ Intelligence Quotient

IQR Interquartile Range

mmHg Millimetres of mercury

mg/dl Milligrams per decilitre

MIT Monoidotyronine mmo/l

Mill moll per Litter

NFCS National Food Consumption Survey

NWU North West University

PHC Primary Health Care

ppm Parts per million

RDA Recommended Dietary Allowance

SA South Africa

SIC Salt Iodine concentrations

SPSS Statistical Package for Social Sciences

Tg Thyroglobin

TSH Thyroid Stimulating hormone

T_3 Triiodothyronine

T_4 Thyroxine

UHDC University of Venda Higher Degrees Committee

uic Urinary Iodine Concentrations UNIVEN

University of Venda USA United States of America wic

Water iodine content WHO World Health Organization

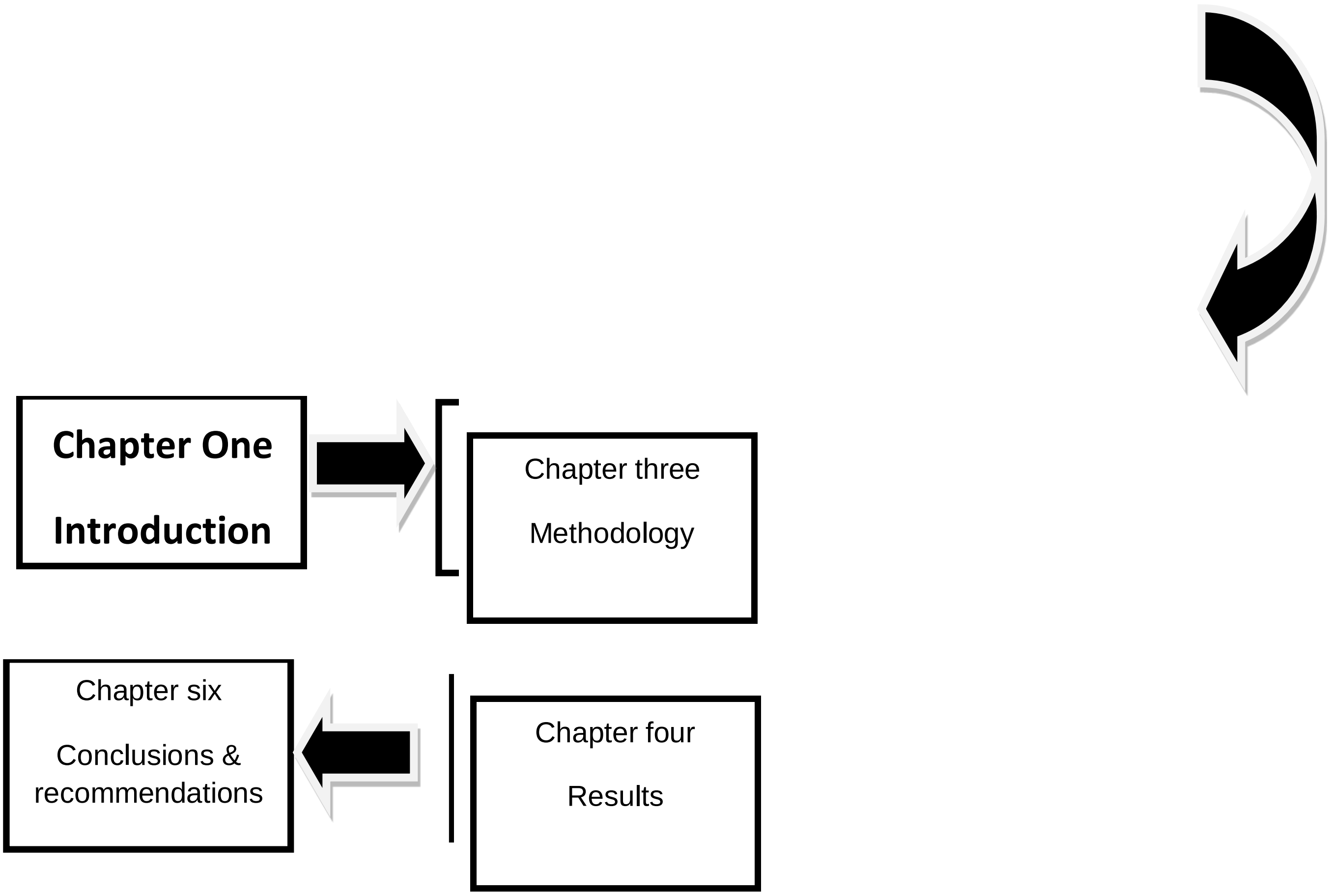
µg/day Microgram per day

µg/l Microgram per litter

CHAPTER ONE

INTRODUCTION

1. OVERVIEW



1.1 BACKGROUND AND MOTIVATION

Iodine is an essential nutrient which is required by humans for the synthesis of thyroid hormones, which are vital for normal growth and development (WHO/UNICEF/ICCIDD, 2007). In pregnant and lactating women, iodine deficiency may lead to disorders that affect those women and their foetuses. The likely effects of the said deficiency are the increased early and late retarded gestation, intellectual disability, endemic cretinism, neonatal hypothyroidism, neonatal hyperthyroitropenemia, increased perinatal and infant mortality, and growth retardation (Boyages and Guttikonda, 2000). It also causes goitres and decreases the production of thyroid hormones that are vital to growth and development. Due to its effect on brain development during gestation, iodine is considered the world's single most significant cause of easily preventable brain damage and mental retardation (Haddow *et al*., 1999).

Children with iodine deficiency can grow up stunted, apathetic, mentally retarded and incapable of normal movement, speech or hearing (Dunn and Van der Haar, 1990). According to Dunn and Van der Haar (1990), iodine deficiency in pregnant women causes miscarriages, stillbirths and mentally retarded children. Furthermore, people living in areas affected by severe iodine deficiency are found to have an intelligence quotient (IQ) of up to 13.5 below those in areas where there is no iodine deficiency (Bleichrodt and Born, 1994).

Iodine deficiency was once considered a minor problem as it caused minor complications such as goitre (an unsightly but seemingly benign cosmetic blemish) (Dunn and Van der Haar, 1990). Due to the advancement in science and technology development, it has been discovered that iodine deficiency is the most common preventable cause of mental handicap in the world today (Stevens and Lowe, 1995). This constitutes a threat to economic development in many countries, including those in Europe (Stevens and Lowe, 1995). The World Health Organization (WHO, 2017) recommends that infants be exclusively breastfed until the age of six months. This is because their iodine intake is solely based on the iodine concentration of the mother's breast milk. Consequently, if the mother's iodine content or levels are low, her offspring is negatively affected. In countries with a good supply of iodine, for example, the breast-milk iodine concentration (BMIC) typically ranges from 150 to 180 µg/L (WHO, 2010). As such, a breastfeeding mother is more likely to supply her infant with enough iodine to meet the Recommended Dietary Allowance (RDA) of 110 µg (WHO, 2017). It should be noted that infants are at high risk for Iodine Deficiency (ID) because their requirements (per kilogram body weight) for iodine and thyroid hormones are much higher than at any other time of their life span (Anderson *et al*., 2010). In this case, the ID becomes significant during gestation when the daily iodine intake falls below 100 µg (Glinoer, 2004).

If blood thyroxine (T_4) and its active derivative triiodothyroidism (T_3) fall, many organs fail to function optimally, and the classical symptoms of hypothyroidism develop (Biggs *et al*., 2011). The impact of hypothyroidism on pregnancy include, *inter alia*, spontaneous miscarriage, still birth, peri-natal death and stunted growth (Biggs *et al*., 2011).

Iodine requirements are increased by more than 50% during gestation due to an increased need for the thyroid hormone (Zimmermann, 2009). Maternal thyroxine (T4) crosses the placenta before the onset of foetal thyroid function, and the normal amounts of thyroid hormones are needed for neuronal migration and the myelination of the foetal brain (Zimmermann, 2008). Even the mild maternal thyroid insufficiency, either subclinical hypothyroidism (elevated thyrotropin (TSH) with the normal free T4 (FT4) or isolated hypothyroxinemia (normal TSH with lower FT4) may impair the foetal neurodevelopment

(Haddow *et al*., 1999; Morreale de Escobar *et al*., 2000; Pop *et al*., 2003; Zoeller, 2003). Worldwide, moderate to severe ID is the frequent cause of maternal and foetal thyroid hormone deficiency and is, therefore, one of the most common causes of preventable mental retardation (WHO/ UNICEF/ICCIDD, 2007).

The thyroid disorders are the most common endocrine disorders in women of childbearing age, and they complicate approximately 1 to 2% of the pregnancies (Glinoer, 2004). Hormonal changes and the increase metabolic demands during pregnancy produce complex alterations in the thyroid hormone concentrations (Casey and Leveno, 2006). The changes include the increased renal iodine losses, an oestrogen induced increase in thyroxine-binding globulin concentration, an increase in the tissue volume of T_4 degradation and transport. These changes are dependent on the stage of gestation (Glinoer, 2003).

Although studies on the BMIC in other countries have been conducted (Bouhouch, 2013; Dorea, 2002; Molla, 2013), little is known about the iodine status of lactating women and infants, and the breast milk iodine concentration in the Vhembe and Mopani districts.

1.2 PROBLEM STATEMENT

Maternal ID has long been recognised to increase the risk of miscarriage and stillbirth. It is also associated with the mental retardation and developmental delays in the affected mothers' offsprings (Dunn and Delange, 2001). Mild or subclinical maternal hypothyroidism during pregnancy may lead to impaired mental development in the offspring (Haddow *et al*., 1999). The severity of maternal hypothyroidism is inversely correlated with the offspring's IQ (Klein *et al*., 2009).

The foetus is dependent on maternal thyroxine for normal brain development because the foetal thyroid gland development and hormone production are relatively delayed during gestation (Becker *et al*., 2006). The production and transfer of thyroxine (T4) and iodide to the foetus requires that women consume an additional 50 - 100µg of iodine during pregnancy in order to maintain euthyroidism (Delange, 2004).

The ID is endemic in some areas in South East Asia and most of the West and Central Africa, especially those areas where soil levels are chronically depleted as a result of flooding and erosion (WHO/UNICEF/ICCIDD, 2007). In most of these areas, the universal fortification of the table salt was introduced as an inexpensive and safe method for the prevention and control of IDD. In spite of these efforts, mild to moderate deficiency has remained or re-emerged in countries previously considered iodine sufficient such as Cambodia, potentially due to the incomplete fortification and/or issues related to poor coverage or access of adequately iodised salt (Bath *et al*., 2013).

Most studies (Mabapa *et al*., 2014; Zimmermann *et al*., 2007; Mabapa *et al*., 2013/14) assessed the iodine status of populations using the primary school children and pregnant women's UIC in the Vhembe and Mopani Districts. Little is known about the iodine content of breast milk in the Vhembe and Mopani Districts. Thus, there is a need to determine the iodine concentration levels of breast milk in these two districts given this gap in literature.

1.3 AIM

The aim of this study was to determine the iodine status of lactating mothers and infants aged 0 to 6 months in the Vhembe and Mopani Districts.

1.4 OBJECTIVES

The following constituted this study's objectives:

1.4.1 To determine the iodine concentration in breast milk.

1.4.2 To determine the lactating mothers' urinary iodine concentration.

1.4.3 To determine the urinary iodine concentration of exclusively and non-exclusively breastfed infants.

1.4.4 To determine the household salt's iodine concentration.

1.4.5 To determine the iodine concentration in household drinking water.

1.4.6 To compare the BMIC and UIC of infants and mothers in the two districts.

1.5 SIGNIFICANCE OF THE STUDY

It is anticipated that the findings of this study may help the Department of Health Authorities and other interested institutions and organisations as well as policy makers in understanding the iodine status of lactating mothers and infants in the Vhembe and Mopani Districts. Furthermore, this study may provide baseline information for future studies concerning the iodine status among lactating women and infants in the country.

1.6 STRUCTURE OF THE DISSERTATION

Chapter 1 provides the background information on breast milk iodine concentration, the urinary iodine levels of lactating mothers and infants, and the iodine nutrition knowledge. It also presents the aim, objectives and this study's problem statement and its significance.

Chapter 2 reviews literature on iodine nutrition world-wide and, South Africa in particular.

Chapter 3 describes the research methodology used to collect data for this study.

Chapter 4 deals with the results of this study. These include the BMIC, the mothers and infants' UIC, iodine in household salt, water iodine concentration and the correlation between the mothers and infants' UIC and BMIC.

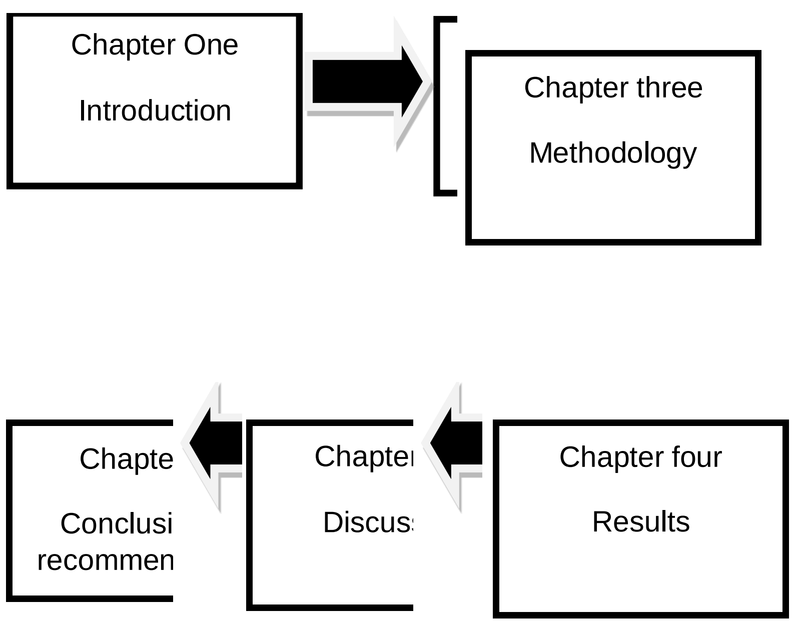
Chapter 5 discusses the results of this study obtained from lactating mothers and their infants.

Chapter 6 concludes this study, and provides its recommendations.

CHAPTER TWO

LITERATURE REVIEW

2. OVERVIEW



2.1 LITERATURE REVIEW

Iodine is an essential element for the production of thyroid hormones, triiodthyronine (T_3) and thyroxine (T_4). These hormones are essential for the normal physical and mental development (Cheryl *et al*., 2006). Lactating and pregnant women require more iodine for foetal brain development than non-lactating and non-pregnant women (Delange, 2004). The ID during infancy and pregnancy may impair growth and the neurodevelopment of the offspring, and at the same time increasing the infant mortality rate. Iodine nutrition during pregnancy and lactation has become an important public health concern because of the deleterious impact of the ID on brain development during foetal and early postnatal life. There is a consensus on the need to take action to eliminate the ID in countries where goitre or even cretinism are prevalent. In regions where dietary iodine intake is borderline (50 – 100µg/day), it may be necessary to increase the dietary supply during pregnancy (Smyth, 1997).

2.2 FUNCTIONS OF IODINE IN THE BODY

Iodine is the essential substrate for the synthesis of the thyroid hormones T3 and T4 (Guthrine and Picciano, 1995). The thyroid hormones L-thyroxine and 3,5,3'-triiodo-L-thyronine are among the fundamental factors that contribute to the normal development of the central nervous system through genomic and non-genomic actions in neurons and glial cells. The thyroid hormones are iodised amino acids, released in the thyroid gland by the TSH induced proteolysis of the iodised thyroglubulin. Thus, iodine is an essential component of the thyroid hormones. The amount of iodine intake per day, which varies according to the individuals' age and physiological state is, therefore, crucial for the thyroid gland to produce the adequate amounts of the thyroid hormones. In particular, the iodine intake is fundamental during gestation and lactation because in these developmental periods, the mother is the only source of TSH, T4 and iodine for the foetus and the breastfed neonate (Berbel *et al*., 2007).

Once taken up from the blood system by the thyroid cells, iodine is released in the thyroid glands colloid where it is oxidised by the hydrogen peroxide derived from the thyroid peroxidise system. It is then incorporated into the tyrosine of the thyroglobin (Tg) to form monoiodotyrosine (MIT) and diiodotyrosine (DIT). When the DIT molecules are coupled with another DIT, the tetraiodotyrosine or T4 is formed. When the MIT and DIT are coupled, the T3 is formed. The Tg is then taken up by the process of proteolysis. The secretion of T4 and T3 from the thyroid gland into the blood stream is influenced by the TSH, which is stimulated by the thyrotropin releasing hormone (TRH) from the hypothalamus (Guthrine and Pacciano, 1995).

The foetus depends on the maternal thyroid hormones for its development. The reliance is mainly on the maternal T4 as only the small amounts of maternal T3 reach the foetal tissues, and almost all the T3 found in the developing cerebral cortex are generated through the local de-iodisation of the T4, ultimately derived from the maternal circulating T4. Thus, during these earliest periods of human life, it is essential for the mother to produce sufficient amounts of the thyroid hormones for herself and the progeny. To achieve this, pregnant women need to double the recommended normal daily intake of iodine to at least 250µg/d. Furthermore, to meet the neonatal requirements, the iodine intake should remain increased during lactation if the infant is solely breastfed. Recurrently, these increased needs are not met, thus the maternal hypothyroxinemia during pregnancy has received increased attention as the cause of the neurodevelopmental disorders. In addition to the maternal thyroid hormones, the foetus also depends on the mother for its iodine supply to the foetal thyroid, as does the neonatal thyroid during lactation (Berbel *et al*., 2007).

2.3 DIETARY REQUIREMENTS FOR IODINE IN THE BODY

The Food and Nutrition Board of the United States National Academy of Science deemed that the daily iodine intake of 1µg/kg of the body weight is adequate for most adults. The growing children, especially the adolescent females, may require more than this amount (Guthrine and Pacciano, 1995). An iodine intake of 150µg/day was suggested as sufficient for all adults and adolescents (Mahan and Stump, 2012). The recommended dietary allowance (RDA) for pregnant and lactating women was increased to 250 µg. The RDA for both the preschool and school children is 90µg/day and 120 µg/day respectively, while 150 µg/day is for adults, and the pregnant and lactating women should get 250 µg/day (WHO/UNICEF/ICCIDD, 2007).

Table 2.1: Daily iodine requirements for Pre-School children, school children, adolescents, pregnant and lactating mothers.

Group	Daily iodine requirements
Pre-School children (0 t0 59 months)	90 µg
School children (6 to 12 years)	120 µg
Adolescents (above 12 years) and adults	150 µg
Pregnant and lactating mothers	250 µg

As recommended by WHO, UNICEF and ICCIDD (2007).

2.4 INDICATORS OF IODINE DEFICIENCY

Four methods are generally recommended for the assessment of iodine nutrition in populations, vis: median urinary iodine concentrations (UIC), serum TSH, serum Tg and the goitre rate. These indicators are complementary in that the UI is a sensitive indicator of the recent iodine intake (days) and the Tg shows an intermediate response (weeks to months), whereas the changes in the goitre rate reflect the long-term iodine nutrition (months to years) (Zimmermann, 2009; Zimmermann *et al*., 2013; WHO/UNICEF/ICCIDD, 2007).

2.5 SOURCES OF IODINE

Iodine is found in the soil and gets to humans and livestock through food grown from the soil. Unfortunately, iodine is not evenly distributed across the Earth 's crust. About 29% of the world's population lives in areas with iodine deficient soils (WHO/UNICEF/ICCIDD, 2007). These include mountainous regions, areas prone to flooding and those subject to soil erosion and deforestation. The oceans contain adequate iodine, and those eating seafood are less likely to suffer from IDD. However, in some coastal communities, this does not necessarily hold true (Jooste, 2001).

In countries with iodised salt, it is generally and primarily the main dietary source of iodine. In settings where foods are prepared at home, household iodised salt is the major source of iodine. However, in industrialised countries, salt used in processed foods contribute approximately 60 to 80% of the total salt intake (Anderson and Zimmerman, 2012). The dietary iodide from sources such as iodised salt or sea foods is rapidly and near being completely absorbed (greater than 90%) in the stomach and duodenum (Zimmermann, 2011).
Iodine exist in variable amounts in the food and drinking water (Mahan and Stump, 2012). Sea foods such as clams, lobsters, oysters, sardines and other salt water fish are the richest natural source of iodine. Salt water fish contain 300 to 3000 µg/kg, fresh water fish contain 20 to 40 µg, but they are still good sources of iodine. The cow milk and eggs' iodine content is determined by the iodine availability in those animals' diet. The vegetables' iodine content varies according to the iodide content of the soil in which they are grown. But, the iodide content is generally low, and therefore not a significant source of dietary iodine. Iodine also enters the food chain through the iodophors, which are used as disinfectants in the dairy processing and colouring agents, and dough conditioners. The best way to obtain an adequate intake of iodine is to use the iodised salt in food preparation.

2.6 PREGNANCY AND LACTATION

The iodine requirement during pregnancy increases due to the following factors.

A. An increase in maternal T_4 production to maintain the maternal euthyroidism and the transfer thyroid hormone to the foetus early in the first trimester before the foetal thyroid functions.

B. Iodine transfer to the foetus, particularly in later gestation.

C. An increase in the renal iodine clearance.

The average iodine retention of the full term infant is 7.3 µg/ kg (Delange, 1989). The mean retention of a healthy foetus with a weight of 3 kg would be approximately 22 µg/d. The estimated daily foetal iodine retention added to the EAR of 95 µg/d for non-pregnant women would yield an EAR of 117 µg/d. But, this does not take into account the iodine needed to increase the maternal T_4 production to balance the additional urinary losses.

2.7 EFFECTS OF DEFICIENCY THROUGH THE LIFE CYCLE

The ID has multiple adverse effects on growth and development in animals and humans. These are collectively termed the iodine deficiency disorders (IDDs). They result from inadequate thyroid hormone production due to the lack of sufficient iodine.

2.7.1 Pregnancy and infancy

In areas of severe chronic iodine deficiency, maternal and foetal hypothyroxinemia can occur from early gestation onward (Morreale *et al*., 2005). The thyroid hormone is required for the normal neuronal migration, the myelination of the brain during foetal and early postnatal life, and hypothyroxinemia during these critical periods causes irreversible brain damage, with mental retardation and neurological abnormalities (Maccarisson, 1908). The consequences depend on the timing and severity of the hypothyroxinemia. Maccarisson's (1908) original descriptions of cretinism in northern India, delineated the neurological form with the predominantly neuromotor defects, and a myxedematous form marked by severe hypothyroidism and short stature.

His observations were subsequently expanded by authors such as Halpern *et al*. (1994) and Delange (1974). The three characteristic features of the neurological cretinism in its fully developed form are severe mental retardation with squint, deaf mutism and motor spasticity. The mental deficiency is characterised by the marked impairment of the abstract thought, whereas the autonomic and vegetative functions and memory are relatively well preserved, except in the most severe cases. Vision is unaffected, whereas deafness is characteristic. This may be complete in as many as 50% cretins, as confirmed by studies of the auditory brainstem-evoked potentials.

The motor disorder shows the proximal spasticity involvement of the feet and hands as unusual, and their function is characteristically preserved so that most cretins can walk. This may be useful in differentiating cretinism from other forms of cerebral palsy commonly encountered in endemic cases such as the cerebral palsy from birth injury or meningitis. The typical myxedematous has a less severe degree of mental retardation than the neurological cretin. But, it has all the features of severe hypothyroidism present since early life, including the severe growth retardation, incomplete maturation of the features. These include the narsoorbital configuration, the atrophy of the mandibles, puffy features, myxedematous, thickened and dry skin, dry and rare hair, and delayed sexual maturation. In contrast to the general population and neurological cretinism, goitre is usually absent, and the thyroid is usually atrophic (Nordenberg *et al*., 1993). The circulating T_4 and T_3 are extremely low, often undetectable, and the TSH is dramatically high. It may be difficult to differentiate between these two forms of cretinism, that is, cretinism may present itself as a mixed form with features of both (Delange *et al*., 1986; Carta *et al*., 1988). Whether mild-to-moderate, the maternal ID's causes of the subtler impairment of cognitive and/or neurological function in the offspring is uncertain. Two case-control studies in iodine-sufficient women with mild thyroid hypofunction reported the developmental impairment in their offspring. In the United States the IQ scores of the 7 to 9-yr-old children of mothers with subclinical hypothyroidism during pregnancy (an increased TSH in the second trimester) were 7 points lower compared with those from mothers with normal thyroid function during pregnancy (Haddow *et al*., 1999). According to Pop *et al*. (1999), in the Netherlands, the infants' development to 2 years was impaired in children whose women had a free T_4 (FT4) below the 10^{th} percentile at 12 week gestation. This suggests that the cognitive deficits may occur in the offspring even if the maternal hypothyroidism is mild and asymptomatic. However, the maternal thyroid dysfunction was presumably not due to iodine deficiency because they were in iodine-sufficient populations. It is unclear whether the maternal hypothyroxinemia and/or subclinical hypothyroidism occurs in otherwise healthy pregnant women with mild-to-moderate iodine deficiency.

2.7.2 Childhood

1. Cognition

There have been many cross-sectional studies comparing cognition and/or motor function in children from chronically iodine deficient and iodine sufficient areas, including those of Asian and European backgrounds (Boyeges *et al*., 1989). These cross-sectional studies, with a few exceptions, reveal the reduced intellectual function and motor skills in iodine-deficient areas. However, observational studies are often confounded by other factors that affect child development (Sameroff *et al*., 1993). Also, these studies could not distinguish between the persistence in utero iodine deficiency and the effects of the current iodine status.

The two meta-analyses were reported on this issue (Bleichrodt, 1994; Quian *et al*., 1982). The first was done through 21 observations and experimental studies, including a control group of the effects of ID on mental development (Bleichrodt, 1994). Of these, 16 studies were in children, four included adults, and two included infants. The age range was 2 - 45 years. The final meta-analysis included 2214 participants (mainly children), and their IQs were used as the main outcomes measure. The studies were all done in areas of moderate to severe-iodinedeficiency. The IQs of the non-iodine-deficient groups were, on average, 13.5 IQ points higher than those of the iodine-deficient groups. However, the studies included in this analysis were of varying quality. That is, much of their data came from observational studies, and only six of the papers cited were published in peer-reviewed journals. The inclusion criteria for the second meta-analysis (Qian *et al*., 2005) included all studies conducted in China, that compared children (<16 year old) living in naturally Iodine-Sufficient areas with those in severe iodinedeficient areas; 2) children in iodine-deficient areas born before the introduction of the iodine prophylaxis; and 3) children in iodine-deficient areas born after the introduction of iodine prophylaxis. Their IQs were measured using the Binet or Raven's scales. The effect size was an increase of 12.45, 12.3 and 4.8 IQ points respectively, for the iodine sufficient group and the latter two groups, compared with those in iodine-deficient areas. Compared with severely iodine-deficient children, there was an increase of approximately 12 IQ points for children born more than 3.5 years after the iodine prophylaxis was introduced. Although it is stated that the iodine-sufficient control groups were comparable socially, economically and educationally, it is difficult to judge the overall quality of the studies included in this meta-analysis. Despite the clear limitations of the mainly cross sectional data included in these two meta-analysis their overall conclusions are similar (Bleichrodt, 1994; Qian *et al*., 2005). They estimate that populations, particularly that of children with chronic and severe iodine deficiency, experience a mean reduction in the IQ of 12 - 13.5 points. For a child born and raised under conditions of the ID, the iodine treatment at school age is beneficial. Several randomised and controlled trials on school-aged children tried to measure the effect of iodised oil on cognition.

2.8 SOMATIC GROWTH

The severe ID in utero causes cretinism and dwarfism, and the iodised oil given during pregnancy in areas of moderate ID increases birthweight by 100 - 200g (Delong *et al*., 1997and Koutras *at al*., 1973). Less clear is the relationship between the ID and the postnatal growth. Data from cross-sectional studies on iodine intake and child growth are mixed (Bautisa *at al*., 1977; Mason *at al*., 2002), with most studies finding modest positive correlations. In five Asian countries, the household access to iodised salt was correlated with the increased weight-for-age and mid-upper-arm circumferences in infancy (Mason *et al*., 2002). The iodine given with other micronutrients generally does not affect child growth (Moreno-Reyes, 2003). The Iodine status may influence growth through its effects on the thyroid axis. The administration of T_4 to hypothyroid children increases their growth (Hernandez-Cassis, 1995). The thyroid hormone promotes the GH secretion and modulates the effects of the GH at its receptor (Hochberg *et al*., 1990). The IGF-I and IGF binding protein (IGFBP)-3 are also dependent on the thyroid status (Nanto-salonen *et al*., 1993). In humans, the hypothyroidism decreases circulating the IGF-I and IGFBP-3 levels, and the thyroid hormone replacement increases them (Iglesias *et al*., 2001). In Iodine-deficient children, the impaired thyroid function and goitre are inversely correlated with the IGF-I and IGFBP-3 concentrations (Alikapifolu *et al*., 2002). However, in an uncontrollable trial, the oral iodised oil paradoxically decreased the IGF-I and IGFBP-3 concentrations in the Turkish children (Ozon, 2004).

2.9 BREAST FEEDING AND INFANT'S IODINE STATUS

The human infant is sensitive to maternal iodine nutrition mainly during foetal development. Besides showing a high and positive correlation between iodine in urine and breast milk (Nohr *et al*., 1994), the thyroid volume in neonates is also associated with the maternal iodine status and breast milk iodine concentrations (Bohles *et al*., 1993). Beaufrere *et al*. (1993) summarised studies from Europe that show that the countries with the lowest breast milk iodine concentrations also had low iodine values in breast fed infants' urine. Bohles *et al*. (1993) observed that in spontaneous thyroid volume development during the first three months of life, exclusively breastfed infants showed the greatest reduction in comparison to formula fed (with and without iodine supplementation), or partially breastfed infants. Bakker *et al*. (1999) found that in the early days after post-partum, there is a temporarily negative iodine balance in healthy term babies.

Indeed, the urinary iodine excretion in the three days old neonates were greater in breastfed than in bottle fed infants (Smyth, 1999). However, in older breast fed infants, the urinary iodine is more sensitive to dietary intake. The urinary iodine at three months was lower in breast fed than in iodine supplemented formula fed infants (Manz *et al*., 1993). But, no significant difference was found between controls and malnourished (low mid-upper-arm circumference) breast fed infants (Akanji *et al*., 1996). The infants' serum T4 and T3 were significantly higher in breast fed compared to formula-fed term and preterm.

Also, the breast-fed infants whose mothers have the thyroid malfunctions (goitre, graves and hashimoto diseases) showed the serum thyroid hormones comparable to formula fed infants (Cho *et al*., 2000). Furthermore, the long lasting effects of breast-feeding on the thyroid gland was observed after three years (Philips *et al*., 1993). The advantages of breast-feeding are indisputable. However, in iodine nutrition, there is speculation that a “bio chemical imprinting” may operate in breast-fed infants (Philips *et al*., 1993).

2.10 STRATEGIES TO PREVENT OR CORRECT DEFICIENCY DURING PREGNANCY AND LACTATION

For nearly all countries, the primary strategy for the sustainable elimination of iodine deficiency in pregnancy remains the universal salt iodisation (Delange and Zupan, 2007). In countries or regions where a salt iodisation programme covers at least 90% of the households and has been sustained for at least two years, and the median UI indicates iodine sufficiency, pregnant and lactating women do not need iodine supplementation (Caldwell *at al*., 2008). Several countries with long-standing, successful iodised salt programmes – China, Iran and Switzerland, *inter alia*, reported an optimal median UI in pregnant women. Also, in countries affected by mild or moderate ID (Ireland, Germany, Belgium, Italy and Denmark), the thyroid volume increases 15 - 31% during pregnancy, whereas in iodine- sufficient countries (Finland and The Netherlands), there is little or no increase in the thyroid volume during pregnancy (Berghout and Wiersinga, 1998). These data suggest that the effective salt iodisation can provide adequate iodine intake during pregnancy, but iodine-containing supplements taken during the prenatal period may have contributed to the iodine intakes in these studies (Zimmermann and Delange, 2004).

However, the implementation of the USI is not always feasible, and this may result in insufficient access of the iodised salt by women of the child bearing age and those pregnant. When these happen, the supplementary of these groups should be considered. WHO (2007) recommends that countries assess their salt iodisation programmes and then decide whether supplementary is indicated. The highly populated countries should use the disaggregated data and categorise areas of the country according to sub national (region, province, and district) data. To ensure the adequate iodine supply during pregnancy, women should ideally be provided with ample iodine intake (at least 150µg/d) for a long period before conception to ensure plentiful intrathyroidal iodine stores (Moleti *et al*., 2008). In Italy, the thyroid function in pregnant women from a mildly iodine-deficient area who had regularly used iodised salt for at least two years before becoming pregnant was compared with that of women who began using iodised salt when they became pregnant (Moleti *et al*., 2008). The findings suggested that the prolonged use of iodised salt is associated with better maternal thyroid function, possibly due to the greater intrathyroidal iodine stores to draw on during pregnancy.

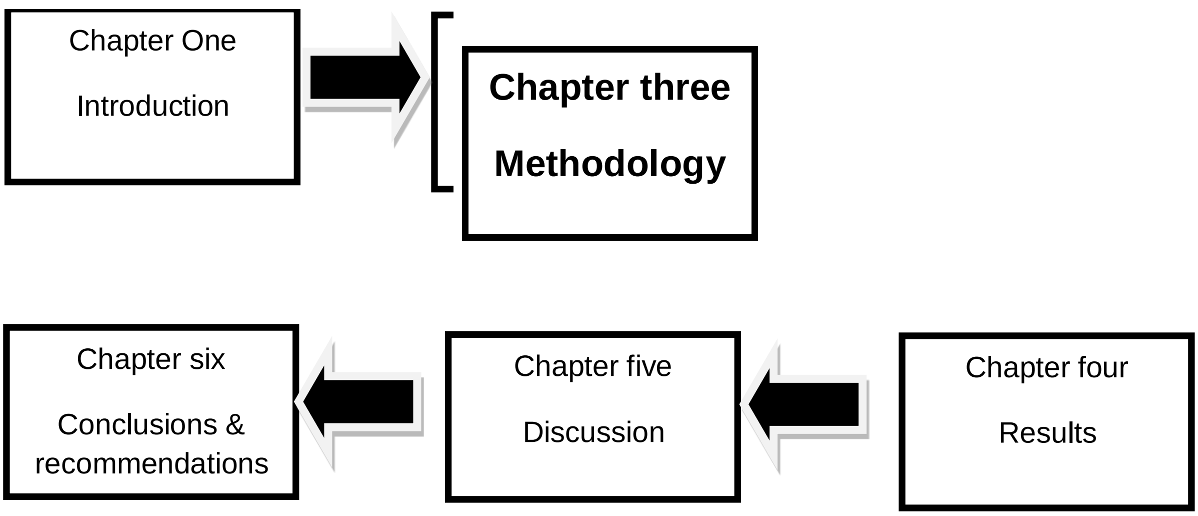
In iodine-deficient countries or regions that have weak iodised salt distribution, that is, in countries or areas where less than 90% of the households use the iodised salt and the median UI is less than 100 µg/L in school children, supplements should be given to pregnant women, lactating women and infants (WHO, UNICEF and ICCIDD, 2007).

Worldwide, the most effective strategy to control iodine deficiency has been found to be salt iodisation (Zimmermann, 2009). The success of this salt iodisation depends largely on the level of iodine mandated and the quality of monitoring and enforcement (Harton, 2000).

CHAPTER THREE

METHODOLOGY

3. OVERVIEW



3.1 STUDY DESIGN

The study design was cross-sectional, whereby the researcher aimed at describing the iodine status of lactating women and infants, and the breast milk concentrations and the association between iodine status of mothers and breast milk iodine content in the Vhembe and Mopani Districts. Cross-sectional studies can be thought of as providing a “snapshot” of the frequency and characteristics of a disease in a population at a particular point in time (Leedy and Ormrod, 2014). The research approach was quantitative because the information was collected by quantifiable measures, for example, biochemical variables. According to Schneider *et al*. (2004), this type of data can be used to assess the prevalence of acute or chronic conditions in a population.

3.2 POPULATION AND STUDY AREA

The target population was lactating mothers and infants aged 0 – 6 months in the Vhembe and Mopani Districts of the Limpopo Province. This target group was selected because women and children are vulnerable and at risk of the ID. The iodine status of pregnant and lactating women, and children reflects the iodine status of the population. Furthermore, children are at an important stage of life where nutrition plays an important role in growth and development, and has long lasting effects in later life (Khan, Khattak and Ali, 2010).

The study was conducted in clinics and health centres of the Vhembe and Mopani Districts. These are two of the five districts of the Limpopo Province. The Vhembe District is divided into four municipalities namely Thulamela, Makhado, Musina and Mutale, whilst the Mopani District is divided into six municipalities namely, Baphalaborwa, Greater Giyani, Greater Letaba, Greater Tzaneen and Maruleng. According to the 2011 census, the Vhembe District has a total population of 1,294,722, and 67.2% of this population speaks Tshivenda, 24.8% speaks Xitsonga, 1.3 speaks Afrikaans and 1.6% speaks Sepedi. The Mopani District has an estimated total population of 964 195 and is located within the North Eastern quadrant of the Limpopo Province. It shares borders with the Vhembe to the North and the Capricorn District to the west. The Mopani District is mainly rural and dominated by Xitsonga and Pedi speaking people. The Vhembe District Municipality has 115 clinics in total, 52 in Thulamela, 3 in Musina, 16 in Mutale and 44 in Makhado. The Mopani District Municipality has 41 clinics in total, 21 are in Greater Giyani, 7 in Greater Tzaneen, 6 in Maruleng, 4 in Greater Letaba and 3 from Ba-Phalaborwa (Department of Health, 2017).

3.3 SAMPLING

Sampling is a process of selecting a representative or portion of the designated population (Leedy and Ormrod, 2014). The purpose of sampling is to increase the efficiency of a research study (Schneider *et al*., 2003). According to Barlet *et al*. (2001), a sample is a physical representation of the target population. It should have the property that can identify every single element to include it in the sample.

A list of clinics was obtained from the Department of Health's District Offices. Simple random sampling was used to select clinics where subjects were drawn. Simple random sampling is a sampling procedure which provides equal opportunity for the selection of each element in a population (Katzenellenbogen *et al*., 2003; Schneider *et al*., 2003; Castillo, 2009).

The Vhembe and Mopani Districts were conveniently selected. The Slovin Formula was used to determine the total sample size where; n signifies the sample size
N signifies the total number of clinics, and E
signifies the accepted level of error.

VHEMBE

n= N/ (1 + (N X e^2) n=
115/ (1 + (115 x 0.05^2) n=
115/1, 2875 n= 89

MOPANI

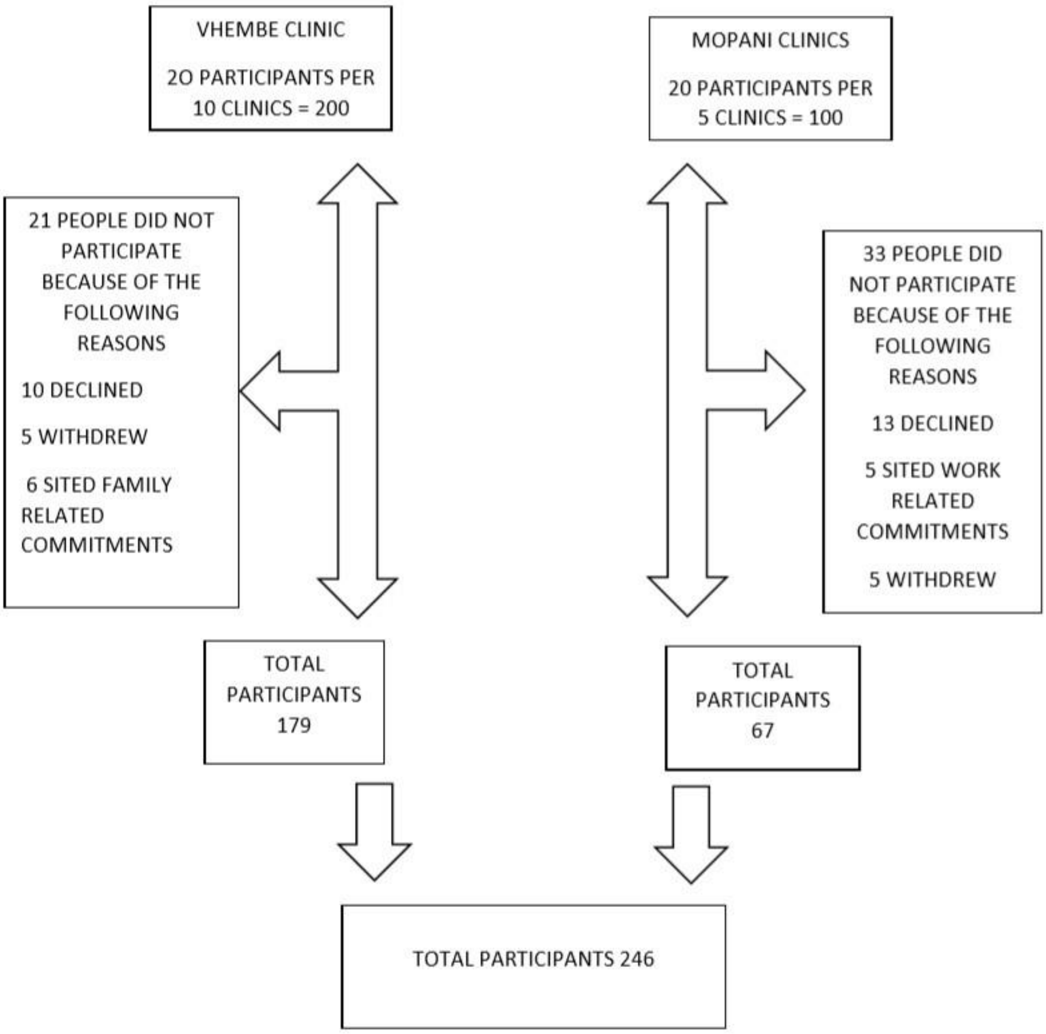
n= N/ (1 + (N X e^2) n=
41/ (1 + (41 x 0.05^2) n=
41/1, 1024 n= 37

The formulae yielded a total of 89 and 37 in the Vhembe and Mopani Districts respectively, and 10% was added in both districts for attrition.

Of the 89 clinics in the Vhembe District, 10 clinics with the highest number of infant-mother pairing attending the growth monitoring were randomly selected. Of the 37 clinics in the Mopani District, 5 clinics with the highest number of the infant-mother pair were randomly selected as well. This researcher chose 15 clinics because of budgetary constraints. From the 15 clinics, 20 infant-mother pairs were conveniently selected from each clinic to make a total of 300 infant mother pairs. See **Figure 3** below.

3.3.1 Recruitment and sample size of the study participants

FIGURE 1



3.4 INCLUSION CRITERIA

This study included all consenting lactating mothers and their infants aged 0 – 6 months. Both exclusive and nonexclusive breastfeeding mothers formed part of the study. Exclusive breastfeeding in this study refers to not feeding an infant anything other than breast milk from birth to 6 months of age, and non-exclusive breastfeeding refers to feeding the infant with breast milk and other stuff such as water and soft porridge from birth to 6 months.

3.5 EXCLUSION CRITERIA

This study did not take into consideration mothers and infants who were on treatment for thyroid related conditions. Lactating mothers and infants who were using any iodine containing supplements were excluded from the study. Mothers who were below the age of 18 years were also excluded from this study.

3.6 LOCATION OF THE RESEARCH

The research was conducted in both clinics and households. In clinics, a questionnaire was used to collect the socio-demographic and dietary practice information. Breast milk, infant urine samples and mother urine samples were also collected at the clinics. Samples of the household salt and water were collected from households.

3.7 SUBJECT RECRUITMENT

First Visit

A list of clinics was obtained from the Vhembe and Mopani Districts' Department of Health. The selected clinics were visited, whereby the aims, objectives and procedures of the study were explained to the PHC managers.

Second Visit

The researcher gave the lactating mothers the recruitment letters explaining the aims, objectives and procedures of the study. He also explained the study verbally to them before distributing the information sheets. (**See Appendix B and C**).

Third Visit

The researcher gave more explanations to mothers who needed more clarity before they could give consent to participating in this study.

3.8 BIOCHEMICAL MEASUREMENTS

The following biochemical measurements were carried out.

3.8.1 Breast Milk

Upon arrival, the study aim, objectives and procedures were fully explained to the mothers in their home language (Tshivenda), and they signed informed consent forms. This researcher also gave them a chance to ask about the study. A 100-ml specimen container with a lit and a wiping cloth were provided to the informants. Breast milk samples (5ml) were obtained by manual expression. The research assistant verbally explained the following procedures to the mother and assisted where necessary:

- Use wet cloth to clean the breast and nipples, as well as hands.
- Do not use any soap or cream to clean your hands, breasts and nipples.
- Manually express a 5ml milk sample from the breast into the milk collection container.
- Collect the breast milk in the collection container provided until the mark shown.

The breast milk was aliquoted into Eppindorf tubes (2-ml) in duplicates and stored at -20 degrees celsius on site and at -80 degrees celsius at UNIVEN until analysis. The breast milk iodine concentration was analysed at the Laboratory of the Human Nutrition of ETH Zurich, Switzerland (Dold *et al*., 2016). Iodine was extracted from the samples using a modified tetramethylammonium hydroxide (TMAH) extraction procedure (Andrey *et al*., 2001). The iodine content in filtered TMAN extracts was measured using the multi-collector inductively coupled plasma mass spectrometer (MC-ICP-MS (Finngan NEPTUNE, Thermo Scientific™ Waltham, MA, USA). The quantification was done using the isotope dilution analysis with ^{129}(SRM 4949C, National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA)). The tellurium (AppliChem, Darmstadt, Germany) was used for mass bias correction of the measured 127/129 intensity ratio according to Russel's law. The iodine concentrations of milk samples were calculated using the dilution factors applied to each milk sample. The method was recently validated at the Human Nutrition Laboratory of ETH, Zurich Switzerland. The following cut-offs in the table below were used although the exact cut-offs were not identified:

Table 3.1 BMIC cut-offs by Henjum et al. (2016)

BMIC Category	Interpretation
<75 µg/L	Deficient
76-100 µg/L	Moderate
101-150 µg/L	Sufficient
151-200 µg/L	Adequate
201-250 µg/L	More than adequate
251- 300 µg/L	Excessive
>300 µg/L	Extremely Excessive

3.8.2 Mother Urine

The spot urine samples (5mL) from mothers were collected using a 40-ml polystyrene urine cup with a screw cap (**Figure 1**). The urine sample was collected between 08h00 and 13h00 and close to the same time as that of the breast milk collection. The professional nurse explained the following procedure to the mother:

- Go to the bathroom
- Wash hands with warm water and them. Do not use soap.
- Void a small amount of urine into the toilet and stop mid-stream.
- Void a small amount into the polystyrene cup until half full.
- Remove the lid from the urine collection cup.
- Transfer the urine from the polystyrene cup into the urine collection cup.
- Close the urine collection cup and screwed on tightly.

- Throw the used polystyrene cup into the bin.
- Wash hands with water and dry them.
- Return the urine sample to the nurse/field worker after collection.

The urine samples were aliquoted in 2.2-3 ml micro tubules (Eppendorfs) with safe lock and packed in storage bags. The plastic pasteur pippette (3ml) were used for aliquoting, 1 pipette per subject. The samples were stored at -20 degree celsius on site and kept refrigerated at –80 degrees celsius until the day of analysis.

The iodine in urine was measured in duplicates with the use of Sandell-Kolthoff method (Sandell & Kolthoff, 1934). The analysis of the iodine concentration of urine was carried out in ETH Zurich. The Sandell-kolthoff uses the Pino modification of the reaction with spectrophotometric detection (Pino *et al*., 1996; WHO/UNICEF/ICCIDD, 2007). Before the spectrophotometric measurement could be used, the urine samples must be treated to destroy organic matter and other substances in it that could interfere with the rate of the reaction.

The laboratory successfully participates in the programme to ensure the quality of Urine Iodine Procedures (EQUIP, US. Centers for Disease Control and Prevention, Atlanta, GA, USA). **Table 3.2** below indicates the cut-offs that were used in this study.

Table 3.2 UIC cut-offs of lactating mothers (WHO/UNICEF/ICCIDD, 2007)

UIC categories	Interpretation
< 100 µg/ L	Insufficient
100 - 250 µg/ L	Adequate
250–499 µg/L	More than adequate
> 500 µg/L	Excessive

3.8.3 Infant Urine

To collect urine samples from infants, the mother was asked to use special infant urine collection bag (**Figure 1**) with an opening to be placed over the baby's genitals, with a sticky film or adherence to the baby's skin and a drainage tab to empty the bag after the baby had urinated into it.

This researcher explained the following steps to the mother when attaching the bag to the males/female babies:

Step1. Thoroughly clean the skin area around the baby's genitals using the provided cleaning wipes.

Step2. Place the opening of the bag over the entire penis or over the labia.

Step3. Carefully fix the bag by adhering the sticky film to the baby's skin. As lively babies can displace the bag, an additional adhesive might be necessary.

After you have attached the bag, put a diaper securely over it. Check the baby often and remove the urine in the bag through the drainage tab or by removing the bag into a special collection bottle provided to you (with syringe if bag has no drainage tab). To prevent the urine leakage, the bag should be checked regularly if it is still solidly fixed or each time when the mother changes the baby's diaper. The bags are safe for the baby. The collection bags are commonly used for urine collection from infants and have been used in many infant studies before. The worst thing that might happen is that the bag leaks or leaves red marks on the baby's skin. The urine was aliquoted into 2ml Eppindorf tubes according to the aliqoutting scheme. The samples were stored at -20 degrees Celsius on site and -80 degree at the laboratory at UNIVEN until the day of data analysis. The same analytic procedure described in the mothers' section was applied for the infants. **Table 3.3** indicates the cut-offs that were used in this study.

Table 3.3 UIC cut-offs of infants (WHO/UNICEF/ICCIDD, 2007)

UIC categories of infants	Interpretation
< 20 µg/L	Severe
20 – 49 µg/L	Moderate
50 – 99 µg/L	Mild
100 – 199 µg/L	Optimum
200 – 299 µg/L	More than adequate
> 300 µg/L	Excessive

Figure 2: Urine collection bag and a polystyrene container.

3.9 SALT

Two table spoons of salt were collected from the households using small plastic bags with ziplocks. The salt samples were stored in a dry cooler temperature and away from the direct sunlight. Salt iodine concentrations were determined at the North-West University (NWU) in Potchefstroom by means of the *iCheck* test method. The procedure is highlighted below.

First step: The salt was diluted with distilled water. The sample per analysis was 1.0mL, the concentration range was >3ppm (mg/kg), and the minimum dilution factor was 1:3.
Second step: The sample was injected in the ready to use reagent vial and if the solution changed colour to purple, it indicated that iodine was present.
Third step: The diluted solution was then analysed using the photometric determination of iodine colorimetric reaction. The units displayed on the *iCheck* test device were in mg/L, and the linear range was set at 1.0-13.0 mg/L, the time per analysis was <10 minutes. **Table 3.4** below indicates the interpretation of the salt analysis.

Table 3.4 Interpretation of iodine content of salt (Kartano *et al.,* 2016).

Interpretations	Iodine level indicators
<5ppm	Non iodized
5-14.9ppm	Inadequately iodized
15-64.9ppm	Adequately iodised
≥65-79.9	More than adequately iodised
≥80ppm	Excessively iodised

3.10 WATER

A 20ml test tube with a screw cap was used to collect water from the households. The tubes were tightly closed and put in ice on site and stored at –80 ºC until taken to the laboratory for analysis. The same procedure used for urine iodine analysis was used to analyse the iodine concentration in water. **Table 3.5** indicates the cut-offs that were used for water in this study.

Table 3.5 Water iodine concentration cut-offs (Liu et al., 2005 and Liu et al., 2015)

WIC categories	Interpretation
< 10 µg/L	Deficient
10 – 49 µg/L	Insufficient
50 - 299 µg/L	Sufficient
> 300 µg/L	Excessive

3.11 SURVEY QUESTIONNAIRE

A questionnaire was used to collect information on the socio-demographic characteristics, iodine nutrition knowledge and the consumption of foods containing goitrogens. The questionnaire was divided into three sections **(See Appendix D).**

Section A was that of the socio-demographic information that included the mother's age, the level of education of the lactating mothers, household income and the infant's age. Section B included breastfeeding practices, the use of iodised salt and the consumption of goitrogenous foods. The last section included iodine nutrition knowledge questions. The questionnaire was researcher administered to cater for the low literacy level, and the local language was used during the interviews.

The questionnaire was developed in English following this study's aims and objectives. Previous studies tracking iodine nutrition knowledge (Jooste *et al*., 2005; NFCS, 2007) were also consulted during the development of the questionnaire. The questionnaire was submitted to the supervisor for inputs and presented to the Department of Nutrition for peer review. An expert in the iodine field was consulted during the development of the questionnaire. The questionnaire was also submitted to the Research and Ethics Committee of the University of Venda for scrutiny. It was pre and pilot tested before data collection.

3.12 PRE-TEST OF INSTRUMENT

Pretesting was carried out by the researcher to test the appropriateness and completeness of the questions. Ten mothers participated in the pre-testing of the instrument. Pretesting is necessary to make sure that both the researcher and the participants understand the questions the same way (Leedy and Ormrod, 2014). It is also an important way to pinpoint problem areas, reduce measurement error, reduce respondents' burdens, determines whether respondents interpret questions correctly and ensures that the order of questions influences the way a respondent answer (Leedy and Ormrod, 2014). During pre-testing in this study, questions were clear and unambiguous to mothers.

3.13 PILOTING

A pilot study is a procedure for testing and validating an instrument by administering it to a small group of participants from the intended population (De Vos *et al*., 2012). During a pilot study, the researcher can recognise and address some of the problems by obtaining information for improving the project, making adjustments to the instrument (De Vos *et al*., 2012). Two clinics were randomly selected and used for the pilot study. Twenty infant-mother pairs participated in the pilot study, that is, ten mother-infant pairs from each clinic. During the pilot study, the feasibility of the project and the data collection procedures were determined. The time taken to complete a questionnaire and sample collection was also assessed. It was considered to be reasonable and tolerable by the mothers. The results of the pilot study informed the researcher about the next step relating to the questionnaire and the study plan. After the pilot study, some changes were made to Section A of the questionnaire, on question 6, which was about marital status. It did not have an option of living with a partner, and hence the addition of this option.

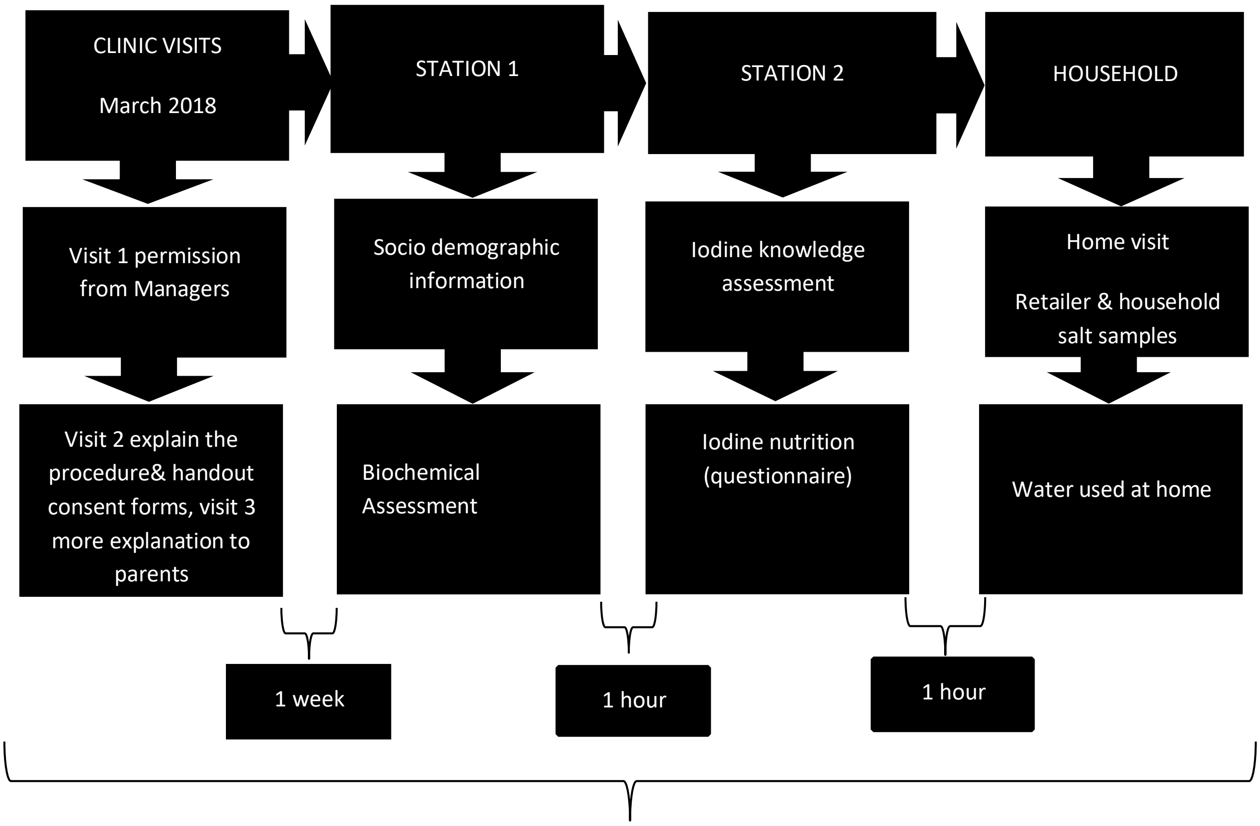
3.14 FIELD WORKERS

A female nutrition graduate was recruited as the research assistant. The reason for using the nutrition graduate was that she was conversant with the research methodology as she had done it during her training. This researcher trained his research assistant during which the methods of data gathering were standardised. The nutrition graduate was responsible for administering questionnaires and collecting samples such as household salt, water, breast milk and the infants and mothers' urine samples.

3.15 PHYSICAL ARRANGEMENTS

A separate room was requested at the clinic where two stations were established. The separate room was used to ensure privacy. In station1, the socio-demographic information as well as the biochemical measurements were taken. In station 2, the Iodine Knowledge assessment and Iodine Nutrition questionnaire was administered to the mothers. Home visits were done where samples of salt and water were collected. **Figure 2** below illustrates the field work plan that was used in this study.

Figure 3: An illustration of the data collection plan.



3.16 QUALITY ASSURANCE

The Potchefstroom's North-West University Laboratory and the Human Laboratory at ETH, Zurich, Switzerland participated in this research to ensure the Quality of Urinary Iodine Procedures. The analytical quality of methods was validated by participating regularly in an external quality assurance programme, namely the EQUIP of CDC, Atlanta, USA.

3.17 INSTITUTIONAL APPROVAL

This study was approved by the Research and Ethics Committee of the University of Venda. Permission to conduct it in clinics was sought from the Provincial Department of Health **(See Appendix A, E, F, G, H, and I)**.

3.18 ETHICAL CONSIDERATIONS

No subjects were sought for this study until the protocol, subject's information sheet recruitment material were approved in writing by the Higher Degrees and Ethics Committee of the University of Venda **(See Appendix E and F).** This study was performed in accordance with the principles of the declarations in Helsinki (2008) and the Good Clinical practice and laws of South Africa. No subject entered the study without signing the informed consent (**Appendix C),** and a full and adequate oral and written explanation of the study, including possible risks. The subjects had the right to withdraw from the study anytime. Data generated from the study was stored in a computer database, and in a manner that maintained subject confidentiality. For data verification and quality control processes, regulatory authorities and/or members of the Higher Degrees and Ethics Committee of the University of Venda might be allowed access to patient data under conditions of strict confidentiality. This was stated in the patient information sheet. The anonymity of the subjects was ensured by using codes instead of names in any publication of data.

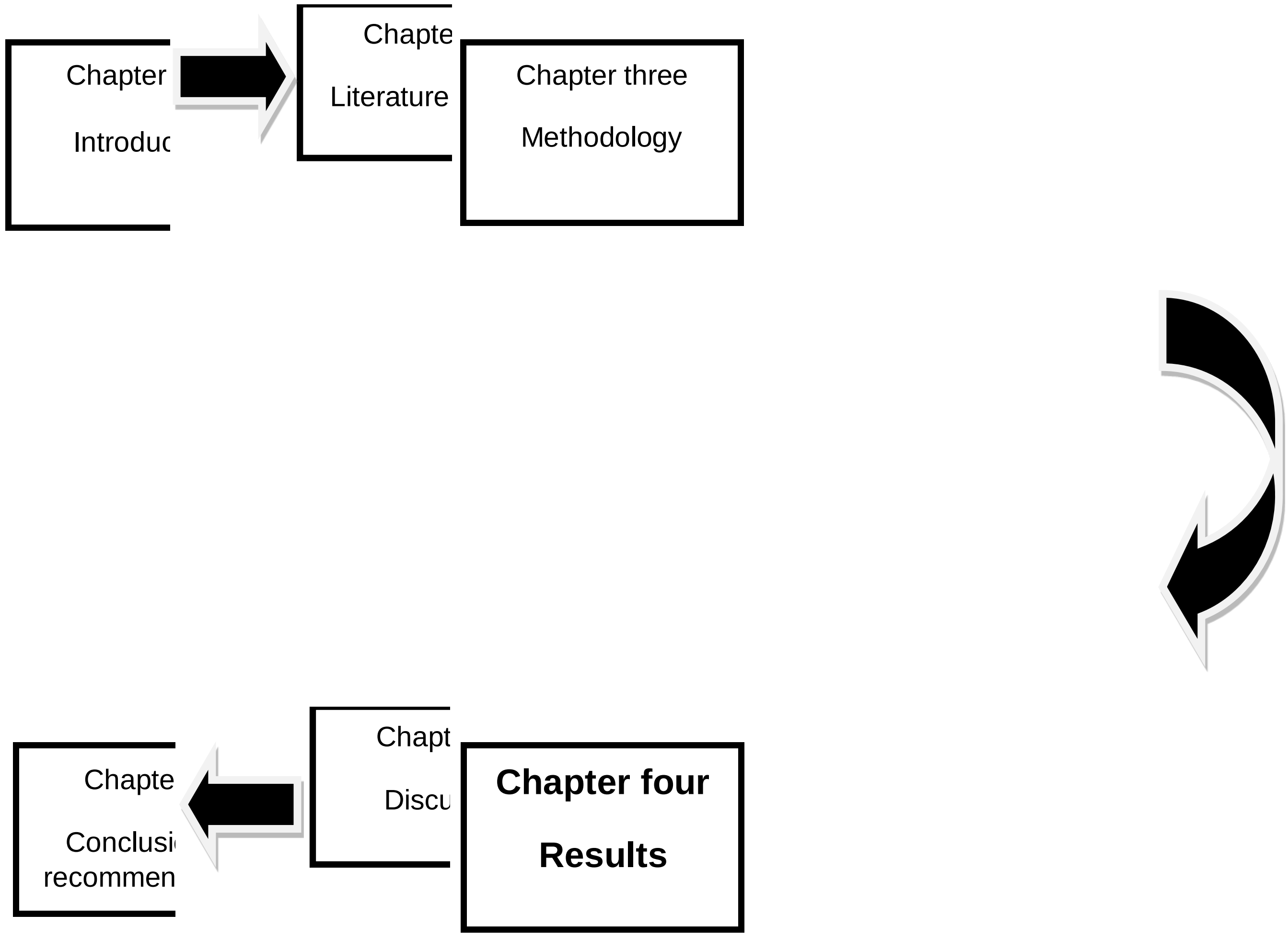
3.19 STATISTICAL ANALYSIS

Data was analysed using the SPSS version 26.0. Data were expressed as medians and interquartile ranges. The categorical data were expressed as frequencies. The Mann-Whitney U test was used (05) to determine differences between groups. The Spearman correlations were performed to determine associations between variables. A p-value <0.05 was considered significant.

CHAPTER FOUR

RESULTS

4. OVERVIEW



This Chapter present the results of the study obtained from 179 and 67 mother-infant pairs in the Vhembe and Mopani Districts respectively. The results are presented in the following order: the participants' socio-demographic information; BMIC; the mothers' UIC; the infants UIC; the salt's iodine content; the water's iodine content; comparisons of variables between the Vhembe and Mopani Districts; the correlation between the BMIC, mothers' UIC and the infants' UIC; the consumption of goitrogenous foods and the iodine nutrition knowledge.

The response rate was 82% in lactating mother-infant pairs. A total of 232 urine samples from lactating mothers and 203 from infants were analysed, representing 77.3% and 67.7% of the samples that were successfully analysed respectively. The other urine samples spilled out during transport before analysis. A total of 219 household salt samples were analysed, representing 73% of the samples that were analysed. Other 27% did not bring salt sample because some households did not have salt available and some could not give salt samples because of religious reasons. A total of 218 drinking water samples were analysed, representing 72.7% of the samples that were successfully analysed. **Table 4.1** below summarises the total number of samples that were collected from the participants.

TABLE 4.1 Actual samples of various groups and variables measured in the study.

Area	Vhembe district	Mopani district	Total number
Samples			
Salt	160	59	219
Water	158	60	218
Infant urine (IU)	152	51	203
Mother urine (MU)	170	62	232
Breast milk (BM)	172	65	237
Questionnaires completed	179	67	246

4.1 SOCIO-DEMOGRAPHIC INFORMATION

4.1.1 Age distribution of the mothers in the Vhembe and Mopani Districts

The participants' age ranged from 18 years to 45 years. The results revealed that 48.9% and 59.7% of the participants were within the 25 - 35 age range in the Vhembe and Mopani Districts respectively. A total of 16.3% and 7.5% in the Vhembe and Mopani Districts ranged between 36 and 45 years of age when data was collected (**Table 4.2**).

TABLE 4.2: Age distribution of mothers in Vhembe and Mopani District

Area	Vhembe district n (%)	Mopani district n (%)
Age of mothers (years)		
18 – 24	62 (34.8%)	22 (32.9%)
25 – 35	87 (48.9%)	40 (59.7%)
36 – 45	29 (16.3%)	5 (7.5%)
TOTAL	178 (100%)	67 (100%)

4.1.2 Age distribution of infants in Vhembe and Mopani District

The age of infants from both districts ranged from 0 to 6 months. The majority in the Vhembe District (24.7%) and few in the Mopani District were two months old when data was collected. The Vhembe District (10.7%) recorded a lower number of infants who were 6 months old than the Mopani District (16.4%) (**Table 4.3**).

TABLE 4.3: Age distribution of infants in the Vhembe and Mopani Districts.

Area	Vhembe district n (%)	Mopani district n (%)
Age of infants (Months)		

Less than one month	34 (19.1%)	5 (7.5%)
2 Months	44 (24.7%)	27 (40.3%)
3 Months	36 (20.2%)	11 (16.4%)
4 Months	21 (11.8%)	8 (11.9%)
5 Months	24 (13.5%)	5 (7.5%)
6 Months	19 (10.7%)	11 (16.4%)
Total	178 (100%)	67 (100%)

4.1.3 Gender of infants in both the districts

A total of 54.5% were girls from the Vhembe District, while 53.7% came from the Mopani District (**Table 4.4**).

TABLE 4.4: Gender distribution of infants in Vhembe and Mopani District.

Area	**Vhembe district n (%)**	**Mopani district n (%)**
Gender of infants		
Boy	81 (45.5%)	30 (44.8%)
Girl	97 (54.5%)	36 (53.7%)
Total	178 (100%)	66 (100%)

4.1.4 Employment status of mothers in Vhembe and Mopani Districts

The type of employment ranged from the health profession to home. The farm workers were recorded as other in the data collection instrument. The Vhembe District had higher percentages of employment in the categories of Health profession, retail industry, security, and cleaning services than the Mopani District. Only 1.1% of mothers were health professionals and 3.4% were employed as cleaners. Also, 87.1% in the Vhembe and 97% in the Mopani Districts were unemployed respectively (**Table 4.5**).

TABLE 4.5 Employment status of mothers in Vhembe and Mopani Districts.

Area	**Vhembe district** n (%)	**Mopani district** n (%)
Employment status		
Health Professional	2 (1.1%)	0
Retail Industry	4 (2.2%)	0
Security	3 (1.7%)	0
Cleaning Services	6 (3.4%)	0
Education	2 (1.1%)	1 (1.5%)
Other	6 (3.4%)	1 (1.5%)
Unemployed	155 (87.1%)	65 (97%)
TOTAL	178 (100%)	67 (100%)

4.1.5 The participants' household income per month

The household income ranged from less than R500 to greater than R6000 in both districts. A total of 53.9% of the participants in the Vhembe and 43.9% in the Mopani Districts had an income within the R1001 to R3500 brackets. Of 178 participants in the Vhembe and 67 in the Mopani Districts respectively, 1.1% and 10.5% had an income of less than R500 respectively (**Table 4.6**).

TABLE 4.6: The participants' household income per month

Area	Vhembe district n (%)	Mopani district n (%)
Household Income		
Less than R500	2 (1.1%)	7 (10.5%)
R500 – R1000	28 (15.7%)	16 (23.9%)
R1001 – R3500	96 (53.9%)	29 (43.9%)
R3501 – R6000	28 (15.7%)	9 (13.4%)
Greater than R6000	24 (13.5%)	6 (9.0%)
TOTAL	178 (100%)	67 (100%)

4.1.6 Level of education of mothers in the Vhembe and Mopani Districts

The results show that 46.1% in the Vhembe and 61.2% in the Mopani Districts had Grade 11 and Grade 12 respectively. The participants with Grade 1 to 7 were 6.7% in the Vhembe District and 4.5% in the Mopani District. All participants attended school (**Table 4.7**).

TABLE 4.7: Mothers' level of education in the Vhembe and Mopani Districts.

Area	**Vhembe district** n (%)	**Mopani district** n (%)
Level of education		
Grade 1 – 7	12 (6.7%)	3 (4.5%)
Grade 8 – 10	45 (25.3%)	13 (19.4%)
Grade 11 – 12	82 (46.1%)	41 (61.2%)
Tertiary Education	39 (21.9%)	10 (14.9%)
TOTAL	178 (100%)	67 (100%)

4.1.7 Mothers' marital status in the Vhembe and Mopani Districts

The Vhembe District had a higher percentage (42.1%) of the married mothers than the Mopani District (29.9%). The majority (Vhembe = 51.7 % versus Mopani = 67.2%) of the mothers were single in both districts and only 0.6% were widowed in the Vhembe District (**Table 4.8**).

TABLE 4.8: Marital status of mothers in Vhembe and Mopani districts.

Area	**Vhembe district** n (%)	**Mopani district** n (%)
Marital Status		
Single	92 (51.7%)	45 (67.2%)
Married	75 (42.1%)	20 (29.9%)
Widowed	1 (0.6%)	0
Divorced	10 (5.6%)	2 (3.0%)
TOTAL	178 (100%)	67 (100%)

4.1.8 Exclusive breastfeeding of mothers in the Vhembe and the Mopani Districts

When mothers were asked if they breastfed exclusively or not, 50.6% in the Vhembe District said yes, and 64.2% in the Mopani District said no (**Table 4.9**).

TABLE 4.9: Exclusive breastfeeding of mothers in the Vhembe and Mopani Districts.

Area	**Vhembe district n (%)**	**Mopani district n (%)**
Exclusive Breastfeeding		
Yes	90 (50.6%)	24 (35.8%)
No	88 (49.4%)	43 (64.2%)
TOTAL	178 (100%)	66 (100%)

4.2 BREASTMILK IODINE CONCENTRATION (BMIC)

A total of 32.2% mothers in the Vhembe District and 14.8% in the Mopani District had a BMIC of less than 75 µg/L, which signifies iodine deficiency. In the Vhembe District, 22.8% of mothers had the BMIC of between 101 to 150 µg/L, whereas in the Mopani District, the percentage was 16.4. Mothers with the BMIC greater than 300 µg/L were 7.6% in the Vhembe District and 11.55% in the Mopani District (**Table 4.10**).

TABLE 4.10 Frequency distribution of BMIC (Henjum *et al*., 2016).

Area	**Vhembe district n (%)**	**Mopani district n (%)**
Categories of BMIC		
<75 µg/L	55 (32.2%)	9 (14.8%)
76-100 µg/L	28 (16.4%)	9 (14.8%)
101-150 µg/L	39 (22.8%)	10 (16.4%)
151-200 µg/L	17 (9.9%)	15 (24.6%)
201-250 µg/L	11 (6.4%)	6 (9.8%)
251- 300 µg/L	8 (4.7%)	5 (8.2%)
>300 µg/L	13 (7.6%)	7 (11.5%)
Total	171 (100%)	61 (100%)

4.3 URINARY IODINE CONCENTRATIONS (UIC) OF MOTHERS

The mothers' UIC was 98.5 µg/L (IQR 57.66 – 154.53µg/L) in the Vhembe District and 126.08 µg/L (IQR 69.90 – 193.85µg/L) in the Mopani District. The results show that the UIC in the Vhembe District was inadequate, while that of the Mopani District's mothers was adequate as recommended by WHO/UNICEF/ICCIDD. About 51% of the lactating mothers from the Vhembe District were iodine insufficient and 45.2% from the Mopani District had the UIC of between 100µg/L and 250 µg/L, whereas 1.2% from the Vhembe District and 3.2% from the Mopani District had the UIC greater than 500µg/L (**Table 4.11**).

TABLE 4.11 The UIC of mothers in the Vhembe and the Mopani Districts.

Area	Vhembe district n (%)	Mopani district n (%)
UIC categories of mothers		
Insufficient (< 100 µg/ L)	87 (51.1%)	24 (38.7%)
Adequate (100 - 250 µg/ L)	63 (37.3%)	28 (45.2%)
More than adequate (250–499 µg/L)	17 (10.1%)	8 (12.9%)
Excessive (> 500 µg/L)	2 (1.2%)	2 (3.2%)
Total	169 (100%)	62 (100%)
Median UIC of mothers in Vhembe and Mopani districts		
UIC (µg/l)	98.5 µg/l (IQR 57.66–154.53µg/l)	126 µg/l (IQR 69.90–193.85µg/l)

4.4 IODINE STATUS OF INFANTS AGED 0 TO 6 MONTHS

The UIC of infants was 220µg/L (IQR 106.67 – 418.43µg/L) in the Vhembe District and 324.94µg/L (IQR 167.96 – 482.66µg/L) in the Mopani District. A total of 1.3% of infants in the Vhembe District and 2% in the Mopani District had the UIC of less than 20µg/L. More than half (52.9%) of the infants from the Mopani District had the UIC greater than 300µg/L as compared to 35.1% from the Vhembe District. Optimum iodine was observed in 25.2% and 21.6% of the infants' UIC from the Vhembe and the Mopani Districts respectively (**Table 4.12**).

TABLE 4.12: Urinary iodine concentration of infants UIC cut-offs of infants (WHO/UNICEF/ICCIDD, 2007).

Area	Vhembe district n (%)	Mopani district n (%)
UIC categories of infants		
Severe (< 20 µg/L)	2 (1.3%)	1 (2.0%)
Moderate (20 – 49 µg/L)	5 (3.3%)	1 (2.0%)
Mild (50 – 99 µg/L)	26 (17.2%)	1 (2.0%)

Optimum (100 – 199 µg/L)	38 (25.2%)	11 (21.6%)
More than adequate (200 – 299 µg/L)	27 (17.9%)	10 (19.6%)
Excessive (> 300 µg/L)	53 (35.1%)	27 (52.9%)
Total	151 (100%)	51 (100%)
Median UIC of infants in Vhembe an I Mopani districts		
UIC infants (µg/L)	220µg/L (IQR106.67–418.43µg/L)	324.94µg/L (IQR 167.96–482.66µg/L)

4.5 FREQUENCY DISTRIBUTION OF SPOT URINARY IODINE CONCENTRATIONS OF LACTATING MOTHERS AND THEIR BREASTFED INFANTS, AND THE BREASTMILK IODINE CONCENTRATIONS IN µg/l.

A total of 14% of the mothers had the BMIC of less than 50 µg/l in the Vhembe District, whereas in the Mopani District, the percentage was 8.2. This signifies insufficient iodine intake. The UIC of infants below 50 µg/l was 3.3% in the Vhembe District and 4% in the Mopani District. A third (33.3%) of mothers in the Vhembe District and 41% in the Mopani District had the BMIC of between 100 to 199 µg/l, which denotes adequate iodine intake. A total of 35.1% of infants in the Vhembe District and over a half (52.9%) in the Mopani District had the UIC of 300 to 399 µg/l, which signifies excessive iodine intake (**Table 4.13**).

TABLE 4.13: Frequency distribution of spot urinary iodine concentrations of lactating mothers and their breastfed infants, and breastmilk iodine concentrations in µg/L.

Area	**Vhembe district**			**Mopani district**		
Categories of indicators	**BMIC n(%)**	**UIC mothers n(%)**	**UIC infants n(%)**	**BMIC n(%)**	**UIC mothers n(%)**	**UIC infants n(%)**
<50 µg/L	24(14%)	0	5 (3.3%)	5(8.2%)	0	2 (4%)
50-99 µg/L	58(33.9%)	0	26 (17.2%)	13(21.3%)	37 (59.7%)	1 (2%)
100-199 µg/L	57(33.3%)	125 (74%)	38 (25.2%)	25(41%)	15 (24.2%)	11 (21.6%)
200-299 µg/L	20(11.7%)	24 (14.2%)	27 917.9%)	11(18%)	8 (12.9%)	10 (19.6%)
300-399 µg/L	6(3.5%)	17 (10.1%)	53 (35.1%)	7(11.5%)	0	27 (52.9%)
400-499 µg/L	3(1.8%)	0	0	0	0	0
500-599 µg/L	2(1.2%)	3 (1.8%)	0	0	2 (3.2%)	0
>600 µg/L	1(0.6%)	0	0	0	0	0
Total	**171(100%)**	**169 (100%)**	**149 (100%)**	**61(100%)**	62 (100%)	51 (100%)

4.6 UIC DISTRIBUTION OF INFANTS BY AGE

When the UIC of infants was compared according to age (in months), the results revealed a decrease in the UIC from 0 to 6 months. The age of infants was divided into two categories namely, 0 – 3 months and 4 – 6 months. For the age group 0 - 3 months, in the Vhembe District, the UIC of infants was 222.8 µg/l (IQR107.1 – 411.3µg/l), whereas in the Mopani District, the UIC of infants was 358.5 µg/l (IQR 178.9 – 484.7 µg/l). For the age group 4 -6 months, the UIC of infants was 190.9 µg/l (IQR 82.7 – 382.9 µg/l) and 309.5 µg/l (IQR 153.6 – 508.4 µg/l) in the Vhembe and the Mopani Districts respectively. The results suggest a decrease of the UIC (**Table 4.14**).

Table 4.14: UIC distribution of infants by age.

Area	**Vhembe district**		**Mopani district**	
Age of infants	**0-3months**	**4-6months**	**0-3months**	**4-6months**
UIC category of infants				
Severe (< 20 µg/L)	2 (2%)	1 (1.9%)	1 (3.1%)	0
Moderate (20 – 49 µg/L)	3 (3%)	4 (7.7%	1 (3.1%)	0
Mild (50 – 99 µg/L)	16 (16.2%)	10 (19.2%	1 (3.1%)	1 (5%)
Optimum (100 – 199 µg/L)	24 (24.2%)	13 (25%	6 (18.8%)	5 (25%)
More than adequate (200 – 299 µg/L)	18 (18.2%)	7 (13.4%	6 (18.8%)	4 (20%)
Excessive (> 300 µg/L)	36 (36.4%)	17 (32.7%)	17 (53.1%)	10 (50%)
UIC (µg/L)	222.8 µg/l (IQR 107.1 – 411.3 µg/l)	190.9 µg/l (IQR 82.7 – 382.9 µg/l)	358.5 µg/l (IQR 178.9 – 484.7 µg/l)	309.5 µg/l (IQR 153.6 – 508.4 µg/l)

4.7 DISTRIBUTION AND CONCENTRATION OF HOUSEHOLD (HH) SALT IN VHEMBE AND MOPANI DISTRICTS

The iodine concentration of the household salt in the Vhembe District was 14.68 ppm (IQR 6.39 - 37.10 ppm) and 16.82 ppm (IQR 6.53 - 40.40 ppm) in the Mopani District. **Table 4.15** below presents the results of the household salt iodine concentration in the Vhembe and the Mopani Districts. Of 156 salt samples analysed from the Vhembe District, 42.9% of them had iodine concentration of 15-64.9 ppm as compared to 32.2% in the Mopani District. Almost half of the salt samples from the Vhembe District (49.4%) and the Mopani District (49.2%) had iodine concentration of 5-14.9 ppm. In the Vhembe District, 0.6% of the salt samples had iodine of less than 5 ppm (**Table 4.15**).

TABLE 4.15: Salt Iodine Concentration (SIC).

Area	Vhembe district n (%)	Mopani district n (%)
Household SIC		
Non Iodised (<5ppm)	1 (0.6%)	0
Inadequately Iodised (5–14.9 ppm)	77 (49.4%)	29 (49.2%)
Adequately Iodised (15–64.9ppm)	67 (42.9%)	19 (32.2%)
More than adequately Iodised (65–79.9 ppm)	2 (1.3%)	7 (11.9%)
Excessively Iodised (>80 ppm)	9 (5.8%)	4 (6.8%)
Total	156 (100%)	59 (100%)
Median Iodine concentration of HH salt		
Iodine concentrations of HH salt	14.68ppm (IQR 6.39 - 37.10ppm)	16.82ppm (IQR 6.53 - 40.40ppm)

4.7.1 Iodine concentrations of salt by appearance

When the iodine concentrations of coarse and fine salts were compared, the results showed that there was a significant difference between the coarse and fine salts in the Vhembe District (p=0.000) and the same results were obtained in the Mopani District (p=0.001). Coarse salt had lower concentrations of iodine than fine salt in both the Vhembe (6.5 ppm *vs* 25.3 ppm) and the Mopani Districts (6.73 ppm *vs* 27.5 ppm) districts (**Table 4.16**).

TABLE 4.16: Comparisons of salt iodine concentrations by appearance.

Area	Vhembe district median (IQR)	P-value	Mopani district median (IQR)	P-value
Salt appearance		0.000		0.001
Fine Salt	25.3ppm (IQR 12.7–41.7ppm)		27.5ppm (IQR 7.9–70.1ppm)	
Coarse Salt	6.5ppm (IQR 6.1–11.6ppm)		6.73ppm (IQR 5.9–16.8ppm	

4.8 IODINE CONCENTRATION OF DRINKING WATER (WIC)

The iodine concentration of the household drinking water in the Vhembe District was 13 µg/L (IQR 3.42– 25.161 µg/L) and 52.77 µg/L (IQR 15.57 – 273.40 µg/l) in the Mopani District. Forty-one percent (41%) of the household drinking water in the Vhembe District and 18.3% in the Mopani District had iodine concentration of less than 10 µg/L, indicating iodine deficiency. Almost half (46.7%) of the household water had iodine concentration ranging from 50µg to 299µg/L, signifying iodine sufficiency (**Table 4.17**).

TABLE 4.17: Iodine concentration of HH drinking water

Area	**Vhembe district n (%)**	**Mopani district n (%)**
Categories of Iodine concentration of HH drinking water		
Deficient (< 10 µg/L)	64 (41%)	11 (18.3%)
Insufficient (10 – 49 µg/L)	69 (44.2%)	21 (35.0%)
Sufficient (50 - 299 µg/L)	22 (14.1%)	28 (46.7%)
Excessive (> 300 µg/L)	1 (0.6%)	0
Total	156 (100%)	60 (100%)

4.9 COMPARISONS OF THE TWO DISTRICTS' MEDIAN BY INDICATORS

The UIC of mothers in the Vhembe District showed a median of 98.5 µg/L (IQR 57.66 to 153.93 µg/L), whereas in the Mopani District, it was 126.08 µg/l (IQR 69.89 to 193.85 µg/l). The water iodine concentration revealed a median of 13 µg/l (IQR 3.42 to 25.16 µg/l) and in the Mopani District, it shoed 16.83 µg/l (IQR 6.53 to 40.40 µg/l) (**Table 4.18**).

TABLE 4.18: Comparisons of median by indicators in Vhembe and Mopani Districts.

Area	**VHEMBE**	**MOPANI**	**P- VALUES**
Indicators of iodine status			
UIC Mother	98.5 (IQR 57.66 – 153.93)	126.08 (IQR 69.89 – 206.71)	0.71
UIC Infant	220 (IQR 106.67 – 418.43)	193.85 (IQR 167.96 – 482.66)	0.004*
WIC	13 (IQR 3.42 – 25.16)	52.77 (IQR 15.57 – 273.40)	0.006*
SIC of HH	14.68 (IQR 6.39 - 37.10)	16.83 (IQR 6.53 - 40.40)	0.720
BMIC	101.4 (IQR 62.9 - 175.1)	154.4 (IQR 92.6 _ 211.8)	0.003

There was a significant association between the UIC of mothers and the UIC of infants in the Vhembe District (r=0.354, p=0.000). In the Mopani District, the results suggest a significant correlation between the UIC of mothers and that of their infants (r=0.376, p=0.008). There was no significant correlation between the BMIC, UIC of mothers and the UIC of infants (**Table 4.19**).

TABLE 4.19: Correlation between BMIC, UIC of mothers and the UIC of infants.

Area Vhembe district			
	BMIC	**UIC of mothers**	**UIC of infants**
BMIC	**r = 1 p - n = 171**	**r = 0.076 p = 0.335 n = 162**	**r = 0.103 p = 0.220 n = 144**
UIC of mothers	**r = 0.076 p = 0.335 n = 162**	**r = 1 p - n = 162**	**r = 0.354 ** p = 0.000 n = 139**
UIC of infants	**r = 0.103 p = 0.220 n = 144**	**r = 0.354 p = 0.000 n = 139**	**r = 1 p - n = 144**
Area Mopani district			
	BMIC	**UIC of mothers**	**UIC of infants**
BMIC	**r = 1 p - n =61**	**r = 0.129 p = 0.344 n = 56**	**r = 0.151 p = 0.294 n = 50**
UIC of mothers	**r = 0.129 p = 0.344 n = 56**	**r = 1 p - n = 56**	**r =0.376** p 0.008 n = 48**
UIC of infants	**r = 0.151 p = 0.294 n = 50**	**r = 0.376** p = 0.008 n = 48**	**r = 1 p - n = 50**

****Correlation is significant at the 0.01 level**

4.10 IODINE NUTRITION KNOWLEDGE

When participants were asked if they know about salt iodisation during the time of data collection, the majority in the Vhembe District (85,4%) said that they did not know, whereas in the Mopani District, only 10.4% said they know about it. The majority of the participants in the Vhembe District (39.9%) used coarse salt, and in the contrary, the majority in the Mopani District used fine salt (47.8%). Only a minority in the Vhembe District (1.7%) checked salt content in food before buying. Roughly 80.6% of the participants in the Mopani District did not check salt content. The majority of the participants in both districts could not identify the salt formula content on food (**Table 4.20**).

TABLE 4.20: Iodine Nutrition Knowledge

Area	**Vhembe district n(%)**	**Mopani district n(%)**
Do you know about salt iodisation		

Yes	26 (14.6%)	7 (10.4%)
No	152 (85.4%)	60 (89.6%)
TOTAL	178 (100%)	67 (100%)
Which salt do you use		
Fine salt	65 (36.5)	32 (47.8%)
Coarse salt	71 (39.9%)	25 (37.3%)
Both	42 (23.6%)	10 (14.9%)
TOTAL	178 (100%)	67 (100%)
Do you check salt content of food before buying		
Yes	3 (1.7%)	13 (19.4%)
No	175 (98.3%)	54 (80.6%)
TOTAL	178 (100%)	67 (100%)
Can you identify the formula (symbol) for salt on food packages		
Yes	23 (12.9%)	3 (4.5%)
No	155 (87.1%)	64 (95.5%)
TOTAL	178 (100%)	67 (100%)

4.11 CONSUMPTION OF FOODS CONTAINING GOITROGENS

The participants were asked questions to find out about their consumption of commonly consumed foods containing goitrogens such as cabbage and sweet potatoes. The questions asked were related to the frequency of consumption of these foods as well as the preparation methods for consuming these foods.

4.11.1 Consumption of cabbage and sweet potatoes by lactating mothers

A total of 37.6% of the participants in the Vhembe District and 55.2% in the Mopani District consumed cabbage once in a month. Only a few participants in the Vhembe District (1.1%) and 3% in the Mopani District consumed cabbage daily. A higher number of participants in the Vhembe District (10.1%) compared to Mopani (1.5%) never consumed cabbage.

In the Vhembe District, 1.1% of the participants consumed sweet potatoes daily as compared to 4.5% in the Mopani District. The participants who never consumed sweet potatoes were 33.7% in the Vhembe District and 17.9% in the Mopani District (**Table 4.21**).

TABLE 4.21: Consumption of foods containing goitrogents by lactating mothers.

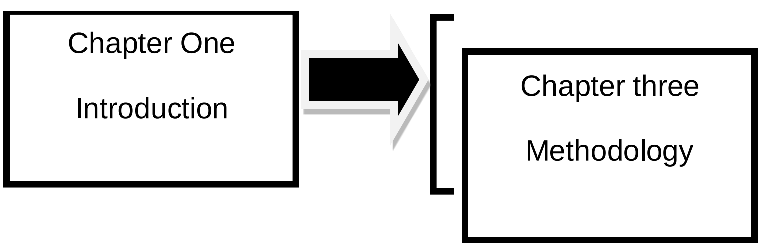
Frequency of consumption	**Vhembe district**	**Mopani district**
	n(%)	n (%)
Consumption of cabbage		
Daily	2 (1.1%)	2 (3.0%)
Once in a week	41 (23.0%)	16 (23.9%)
Once in a month	67 (37.6%)	37 (55.2%)
Twice per month	50 (28.1%)	11 (16.4%)
Never	18 (10.1%)	1 (1.5%)
TOTAL	178 (100.0%)	67 (100%)
How do you eat your cabbage		
Cooked	69 (38.8%)	42 (62.7%)
Uncooked	3 (1.7%)	2 (3.0%)

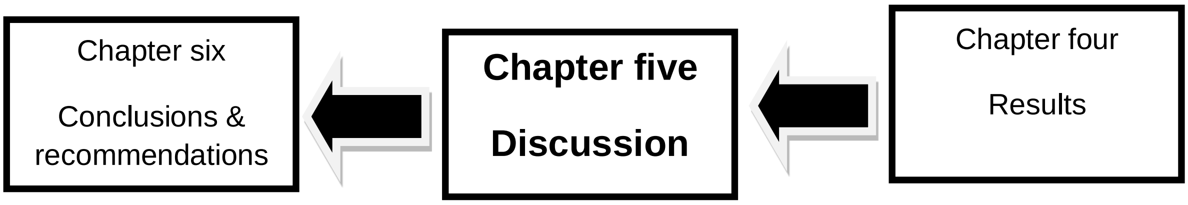
Both	87 (48.9%)	21 (31.3%)
Never	18 (10.1%)	2 (3.0%)
TOTAL	178 (100%)	67 (100%)
Consumption of sweet potatoes		
Daily	1 (1.1%)	3 (4.5%)
Once in a week	8 (23.0%)	7 (10.4)
Once in a month	82 (37.6%)	38 (56.7%)
Twice per month	22 (28.1%)	5 (7.5%)
Never	60 (33.7)	12 (17.9%)
TOTAL	178 (100%)	65 (100%)

CHAPTER FIVE

DISCUSSION

5. OVERVIEW





5.1 SOCIO-DEMOGRAPHIC INFORMATION

The participants were from rural areas of the Vhembe and the Mopani Districts. The majority of them lived in small houses with poor infrastructure, lack of proper sanitation and poor roads. In addition, high unemployment rate was also recorded from the results of this study. Through these observations, it is evident that participants are from poor socio-economic backgrounds. It is important to note that the unemployment rate in the Vhembe (87.1%) and Mopani Districts (97%) was almost three times higher than the national one that is at 29.1% (Quarterly Labour Force Survey of SA, 2019). More than half of the participants in the Vhembe District (53.9%) and almost forty-four percent (43.9%) in the Mopani District had an income of less than R3 500 per month. This monthly income has to be shared among four or more family members. Thus, the results suggest that the participants live below the poverty line. Stats SA's (2019) new report on poverty line that assessed the cost of basic needs found that the "lower-bound poverty line" was R810 per person per month, and the upper-bound poverty line was R1,227 per person per month. Further, the basic income of R561 a month for each person in the household is required to buy nutrient rich foods to stay alive. From these results, it is evident that this is not the case in the two districts studied.

5.2 BREASTMILK IODINE CONCENTRATION (BMIC)

An exact BMIC cut off point has not been specified. However, studies (Dold *et al*., 2017; Henjum *et al*., 2016; Osei *et al*., 2016; Singh *et al*., 2016) show that breastmilk with iodine concentration above 75 µg/l may be considered as an index of sufficient iodine intake. The majority of lactating mothers in this study had the BMIC greater than 75 µg/l. The results suggest iodine sufficiency in lactating mothers. However, a third in the Vhembe District

(32.2%) and 14.8% in the Mopani District had the BMIC of less than 75 µg/l. The BMIC in this study, 101.4µg/l in the Vhembe District and the Mopani District 154 µg/l, is lower than that found in the Potchefstroom's Kenneth Kaunda District (Osei *et al*., 2016, which was 179 µg/l) (IQR 126 to 269 µg/l). At national level, the Breastmilk Iodine Concentration has not been specified. The results also suggest that interventions such as salt fortification may be reaching lactating mothers in rural areas, thus improving their BMIC. On the other hand, some participants in the Vhembe District still use coarse salt, which is voluntarily iodised in SA, thus may explain their BMIC being less than 75µg/l. Mabapa *et al*. (2014) also reported the use of coarse salt in the Vhembe District.

Breastfed infants depend on maternal iodine intake (Yang *et al*., 2017) since adequate iodine concentrations in breast milk is essential for optimal neonatal thyroid hormone synthesis and neurological development in breastfed infants (Henjum *et al*., 2016). During lactation, mothers transfer iodine to their infants by mammary glands, which concentrate iodine breast milk (Yang *et al*., 2017). Infants approximately require 7 µgl/kg of body weight to build iodine pools in their thyroid glands (Zimmermann, 2009).

Based on the BMIC of 101.4 µg/l and a consumption breastmilk of 0.78 l/day at 2 - 5 months (IOM, 2001; Manz *et al*., 2002; Dold *et al*., 2017), the infants from the Vhembe District consume 79 µg of iodine daily whereas in the Mopani District, they consumed 120.4 µg/day of iodine. The results show that in the Vhembe District, infants consume less than 90 µg and 110 µg as recommended by the IOM and WHO respectively for infants below 6 months of age (IOM, 2001; WHO/UNICEF/ICCIDD, 2007). In the Mopani District, infants consume 120 µg/day, which is way above the recommended intake. The results suggest a variation in the BMIC in the Vhembe and Mopani Districts. Congruently, a study conducted by Dold **et al**. (2016) reported that the Moroccan women provided only 23 µg/l per day, whereas the Croatian mothers provided 97 µg/l per day to their infants via breastmilk, covering the daily iodine requirement of their breastfed infants. Sign *et al*. (2016) confirmed that breastmilk iodine concentrations vary in many countries with the same degree of iodine sufficiency or deficiency. Thus, the environmental factors other than iodine depleted soil where crops are grown and iodine concentration in drinking water may influence breastmilk iodine concentration. Iodine also varies due to maternal intake.

The BMIC can be increased by increasing maternal iodine intake (Zimmermann, 2007). In a study in 16 healthy lactating US women, the BMIC was significantly increased by a one-time ingestion of 600µg potassium iodide (456 µg of iodine) with the peak levels at 6/h (Leung *et* *al.*, 2012). According to Semba & Delange (2001); Azizi & Smyth (2009); Dold *et al*. (2017) the BMIC may be a promising indicator of iodine status in lactating women and breastfed infants.

5.3 URINARY IODINE CONCENTRATION (UIC) OF LACTATING MOTHERS

A median urinary iodine concentration above 100 µg/l in lactating mothers is regarded as sufficient, according to the WHO (2007). This study has revealed that the median urinary iodine concentration of lactating mothers in the Vhembe District is 98.5 µg/l (IQR 57.66 – 154.53 µg/l) and 126.08 µg/l (IQR 69.89 – 193.85 µg/l) in the Mopani District. It can be deduced from these results that the UIC of mothers in the Vhembe District denotes iodine deficiency, and sufficiency in the Mopani District. Although the UIC of mothers in the two districts were not significantly different (p=0.71), the Vhembe had a lower UIC reading compared to Mopani. The UIC of mothers in Vhembe is also lower than that of 111µg/l (IQR 67-179 µg/l) found by Osei *et al*. (2016) in the Potchefstroom's Kenneth Kaunda District. Although iodine in water does not meet the daily requirement of an individual, its combination with salt fortification may be the reason for iodine sufficiency in the Mopani District. Mabasa *et al*. (2018) confirmed that the water iodine concentration in Mopani is high.

Urinary iodine is a well-accepted, cost efficient and easily obtainable indicator for the iodine status (WHO/UNICEF/ICCIDD, 2007; Wang *et al*., 2019), since most iodine is absorbed by the body is excreted in through urine (Gibson, 2005). It is considered a sensitive marker of current iodine intake and can reflect recent changes in the iodine status (WHO, 2013; Ma *et al.*, 2018). However, an individual's UIC can vary daily, or even within the same day, the urinary iodine concentration is therefore not useful for the diagnosis and treatment of individuals (Ramussen, Ovesen & Christiansen, 1999; Konig *et al*., 2011; WHO, 2013).

5.4 URINARY IODINE CONCENTRATION (UIC) OF INFANTS

The UIC of infants in the current study was significantly different (p=0.004) between Vhembe (220 µg/l (IQR 106.67 – 418.43 µg/l)) and Mopani (324.94 µg/l (IQR 167.96 – 482.66 µg/l)). The results suggest that lactating mothers consume more than adequate in the Vhembe District and excess in the Mopani District. The UIC of infants was higher than of mothers. Most studies (Osei *et al*., 2016: Dold *et al*., 2017; Yang *et al*., 2017) tracked the UIC of mother-infant pairs and reported similar results as in this study. The excessive iodine intake is a new problem that is emerging and no studies have been conducted in South Africa to consider the consequences of excessive iodine intake. It is evident that the strategies used in this country to eradicate the IDD are effective since Osei *et al*. (2016); Mabasa *et al*. (2018) reported excessive iodine in the SAC and infants.

A higher than normal iodine intake can affect the gland's proper function, leading to an autoimmune disease, goitre, hypothyroidism, and the iodine-induced thyrotoxicosis of thyroid cancer (Biban and Lichiardopol, 2017). There are studies that show that an excess of iodine is a risk factor for the development of thyroid autoimmunity not only for animal models, but also for the human population because iodine can have both a direct (intracellular oxidative stress) and an indirect (activation of proinflammatory cells) phenomena that recruit (immunocompetent cells) effects on the thyrocites (Biban and Lichiardopol, 2017).

Confirming the decrease of the BMIC on the iodine status of the breastfed infants (Mulrine *et al*., 2010), the results show that the UIC of the breastfed infants decreased from a higher concentration to a lower concentration in the 0 - 3 months and the 4 - 6 months respectively, although the UIC was in the adequate range. This study also revealed that the UIC of infants was positively associated with the BMIC and significantly associated with the UIC of mothers in both districts.

5.5 IODINE IN HOUSEHOLD SALT (SIC)

In this study, the iodine concentration of household salt in the Vhembe District was14.68ppm (IQR 6.39 - 37.10ppm) and 16.82ppm (IQR 6.53 - 40.40ppm) in the Mopani District. The results denoted inadequately iodised salt in the Vhembe District and adequately iodised salt in the Mopani District. Almost half of the salt samples from the HH in the Vhembe (49.4%) and the Mopani Districts (49.2%) contained inadequate iodine. The HH, using adequately iodised salt, were 42.9% in Vhembe and almost a third in Mopani District. These percentages are below the international goal of 90% coverage (Jooste *et al*., 2008).

The WHO/UNICEF/ICCIDD (2014) recommended that the iodine concentration in salt should be within the range of 20 – 60 ppm of iodine in order to provide 150 µg of iodine per person per day. This is done because 20% of iodine is lost from production site, another 20% is lost during cooking and the remaining 20% is meant for consumption. The use of adequately iodised salt in the current study is lower than the national coverage of 97% (NFCS-FB-I, 2007). Mabasa *et al*. (2018) also found that 52.5% of the households in Mopani use adequately iodised salt with an iodine concentration of ≥ 15 ppm. Of concern is that almost half of the HH salt in this study and that done by Mabasa *et al*. (2018) were inadequately iodised.

It is noteworthy to mention that the majority of salt samples with inadequate iodine were coarse in this study. Congruently, a total of 47.1% of the HH salts in Mopani were reported to be coarse (Mabasa *et al*., 2018). The results also revealed that the iodine content of fine salt was higher than that of coarse salt. It can be concluded that the iodisation process seems to be generally less effective in the coarse salt than in fine one, possibly because of differences in particle size, impurities or iodising methods (Jooste, 2003).

It can be assumed that salt produces iodising salt at a concentration of more than 20 ppm and this contributes to the elimination of the ID (WHO/UNICEF/ICCIDD, 2001). However, a proportion of the salt examined in this study was clearly not iodised in accordance with the legal requirements. Inadequate iodine intake may result in a variety of disorders, termed the IDD, such as goitre, cretinism, spontaneous abortion, perinatal mortality, and heart failure (Hetzel, 1983; WHO/UNICEF/ICCIDD, 2001; Li & Eastman, 2012). The reasons for not iodising salt in accordance with the legal requirements may be that in South Africa, monitoring and evaluation is not done properly at production sites, thus some salt producers may overiodise and some may under-iodise.

Iodised salt consumption is the best and most cost effective method of preventing the IDD (Kapil, 2004). Salt was chosen as a vehicle for iodisation for the following reasons; it is consumed by a larger proportion of the population, and the cost of iodising salt is extremely low.

5.6 IODINE IN DRINKING WATER

In this study, the median iodine concentration in water (WIC) samples from the Vhembe District was at 13 µg/l (IQR 3.42– 25.161µg/l) and 52.77µg/l (IQR 15.57 – 273.40 µg/l) in the Mopani District. The WIC in Vhembe was lower than that of 46.2µg/l found by Mabasa *et al*. (2018) in the Mopani District. The WIC in Mopani was higher than that of Vhembe, and even higher than that found by Mabasa *et al*. (2018). The WIC in the two studies are lower than that of 117 found in the Northern Cape by the NFCS (2007). The results of this study suggest sufficient iodine in drinking water in the Mopani District and insufficient iodine in the Vhembe District. Gao *et al*. (2014) confirmed the results of this study by concluding that the geographical distribution of iodine in water varies from one place or environment to the other. Although water is a source of iodine, the total contribution of iodine from drinking water would generally not to be considered as adequate in meeting the requirements of infants and lactating mothers (Jooste *et al*., 2008). Iodine in drinking water may serve as an indication of the amount of iodine occurring naturally in the environment (Jooste *et al*., 2008).

According to the Iodine Global Network, iodine ions in seawater are oxidised to the element iodine, which volatises into the atmosphere and is returned to the soil by rain, completing the cycle. However, the iodine cycling in many regions is slow and incomplete, leaving soils and drinking water iodine depleted. Crops grown in these soils are low in iodine, and humans and animals consuming food grown in these soils become iodine deficient. In plant foods grown in deficient soils, iodine concentration may be as low as 10 ppb dry weight compared to 1ppm in plants from iodine sufficient soils.

5.7 IODINE NUTRITION KNOWLEDGE

This study's results reveal that the majority of the participants had limited iodine nutrition knowledge. About 85.4% in the Vhembe District and 89.6% in the Mopani District did not know anything about salt iodisation. Of concern is that the majority of the participants in Vhembe (87.1%) and Mopani (95.5%) could not identify the symbol for salt on the food package. Jooste, Upson & Charlton (2005) arrived at a similar conclusion in a study done among the adult population where they found that the knowledge level of iodine nutrition is low among South Africans, particularly among the low socio-economic groups. This study's results and that of Jooste *et al*. (2005) seem to suggest that the education and promotion aspects of the iodised salt intervention are seriously lacking. Jooste *et al*. (2005) outlined the reasons for this as,

> "Poor iodine nutrition knowledge might be inadequate inclusion of the topic in the school curriculum, little media reporting on the role of iodine in human health, the false notion that iodised salt will automatically solve the entire iodine deficiency problem, the fact that IDD is generally not a 'visible' condition, and the extensive public health focus on infectious diseases such as HIV/AIDS, tuberculosis and malaria. Against this background, it appears unlikely that the public will generate a demand for sufficiently iodised salt until their level of IDD knowledge has improved."

A study conducted by Lisa and Diana (2013) suggested a direct link between nutrition knowledge and food choices. The more the people have knowledge, the better the chances of them making a healthier food choice.

5.8 FOOD CONTAINING GOITROGENS

Goitrogents are naturally occurring substances found in various foods such as cabbage and sweet potatoes, and they have the ability to inhibit iodine absorption (Zimmermann, 2009).
The term goitrogen refers to the dietary substances that interfere with the thyroid metabolism and can aggravate the effect of iodine deficiency (Zimmermann, 2009). Goitrogents are able to disrupt the normal thyroid function by inhibiting the body's ability to use iodine, block the process by which iodine is taken up into the thyroid, inhibiting the actual secretion of the thyroid

hormones and disrupt the peripheral conversion of T4 to T3 (Zimmermann, 2009). Goitrogenic foods such as cabbage contain glucosinolates, and their metabolites compete with the iodine for thyroid uptake, whereas foods such as sweet potatoes contain cyanogenic, and these may be metabolised to thiocyanates that also compete with iodine for the thyroidal uptake (Zimmermann, 2009).

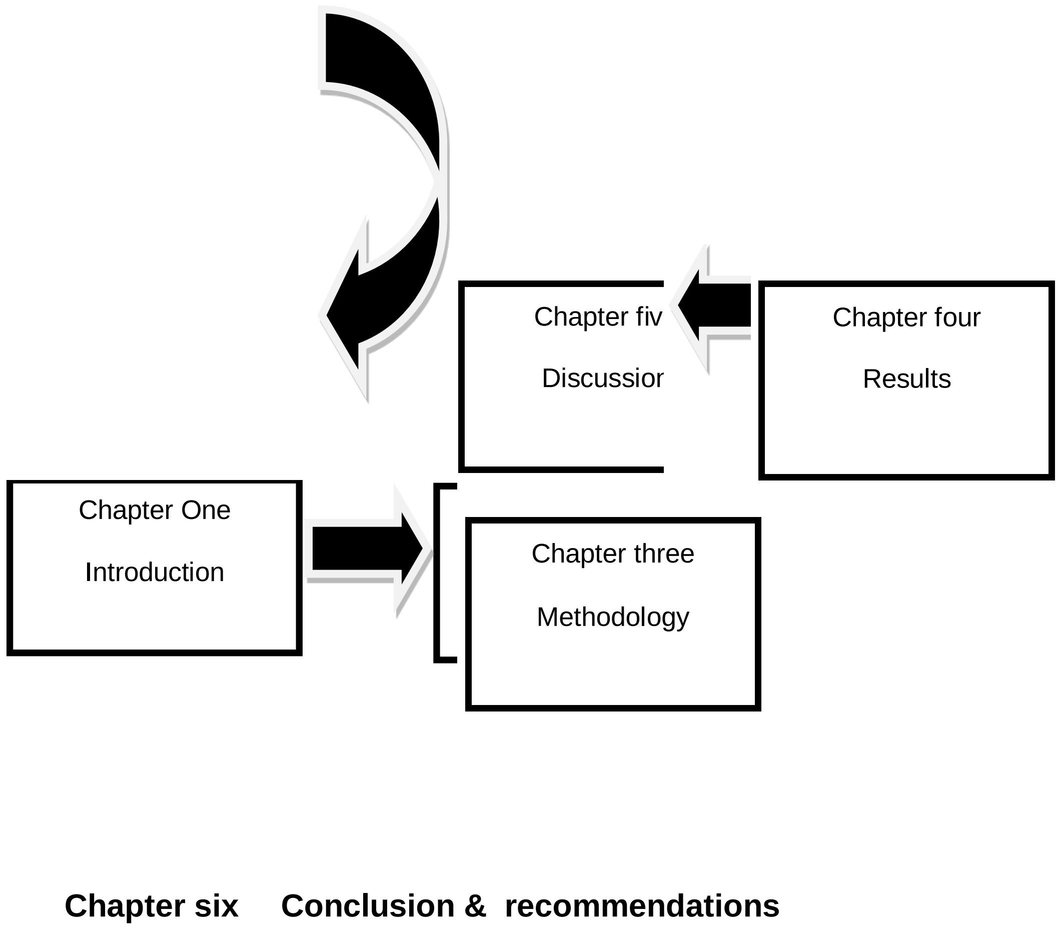
The most consumed goitrogenic foods in this current study were cabbage and sweet potatoes. The majority of the participants reported to consume cabbage in both its cooked and uncooked forms at least once a month. Equally, sweet potatoes were also consumed once a month by most participants. This indicates that the consumption of these foods by the participants is at an insignificant level to inhibit the absorption of iodine. The enzymes involved in the formation of goitrogenic materials in plants can be partially destroyed by cooking heat, allowing these participants to consume these foods cooked.

A conclusion can therefore be drawn that in this study, the consumption patterns of cabbage and sweet potatoes in the two districts did not pose any threat of the metabolism of iodine. This is so because the consumption of these goitrogenic foods is very low. The participants consumed these foods mostly in cooked form.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6. OVERVIEW



6.1 CONCLUSION

6.1.1 Iodine concentration of breast milk

The BMIC in this study signifies iodine sufficiency in both the Vhembe and Mopani Districts. This study's results suggest that the BMIC can be included in studies assessing the iodine status in lactating mothers since the UIC only reflects iodine that was consumed recently. The UIC may under estimate the maternal iodine status if it is not complemented by the BMIC data. From this study's results, the BMIC is proposed as a biomarker of iodine nutrition during lactation. It is a more accurate indicator of iodine status in lactating mothers than the maternal UIC.

6.1.2 Urinary iodine concentration of lactating mothers

The UIC of mothers in the Vhembe District signify iodine deficiency, whereas in the Mopani District, it signifies iodine sufficiency. Some of the lactating mothers in Vhembe use coarse salt, and this may have contributed to the iodine insufficiency as compared to the Mopani District. Although the UIC is considered a good indicator of the iodine status, the results suggest that the median UIC is not an accurate biomarker of iodine intake in lactating mothers.

6.1.3 Urinary iodine concentration of infants

The UIC of infants in the Vhembe Districts signifies more than adequate iodine, and in the Mopani District, it signifies excessive iodine. This may be attributed to the lack of proper monitoring and evaluation at production sites in South Africa. That is, salt producers are not properly monitored and some may under iodise, while some may over iodise, and hence we now observe excessive iodine in children.

6.1.4 Salt iodine concentration

The salt iodine concentration in Vhembe signifies that the household salt was inadequately iodised, whereas in Mopani it signifies adequately iodised salt. The salt iodine concentration of the fine salt was higher than that of the coarse salt. The reason for this may be that coarse salt is not iodised in this country, and it is consumed mostly in the Vhembe District. Although fine salt contains a higher iodine concentration, non-iodised agricultural coarse salt remains the type mostly consumed by households, particularly in the low socio economic communities. This is caused by price differentials between the non-iodised agricultural salt and the iodised fine salt.

6.1.5 Water iodine concentration

Drinking water in the Mopani District had a significant amount of iodine concentration. It can then be concluded that drinking water there is a significant potential source of iodine intake in vulnerable groups.

6.1.6 Iodine nutrition knowledge

The lactating mothers in this study as elsewhere in the country were generally unaware of the iodised salt and the dietary sources of iodine.

6.1.7 Consumption of foods containing goitrogens

The consumption patterns of sweet potatoes and cabbage in the study areas did not pose a threat to the iodine metabolism of the participants. This is because the frequency of consumption was low, and the food is mostly cooked before consumption, and there was no existing ID.

6.1.8 Comparison of BMIC, UIC of lactating mothers and UIC of infants in Vhembe and Mopani Districts

The BMIC, UIC of infants and water iodine concentration were different in the two districts. The variations may be attributed to the geographical locations of these two districts. Although variations were observed, the UIC of children show excessive iodine, which is a problem in South Africa since no studies have been done to determine the consequences of excessive iodine in the human body.

6.2 RECOMMENDATIONS

- This study recommends that the BMIC be used as a biomarker for iodine during lactation. It also recommends that more studies be done in the province and the country at large to assess the breast milk iodine concentration among lactating mothers.
- It recommends that a study be carried out that investigates the actual causes of iodine excessiveness in infants aged 0 – 6 months in the Mopani District and elsewhere in the country. A regular monitoring of iodine status is necessary to detect not only low, but also excessive intake of iodine as demonstrated by the high median UIC of infants in this study.
- This study further recommends that there should be nutrition education and awareness campaigns in the Vhembe and Mopani District on the use of iodised salt and its benefits.
- The coarse salt meant for human and animal consumption should be iodised and regulated by the iodised salt regulations. A provincial strategy should be drafted to overcome the many weaknesses of the salt iodisation programmes in this province in order to achieve the goal of household iodised salt coverage of 90%.
- Drinking water in the Limpopo Province, particularly in the Mopani District should be considered as a significant source of dietary iodine.

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APPENDIX A: LETTER TO REQUEST PERMISSION

The Department of Health
Limpopo Province

Re: REQUEST FOR PERMISSION TO CONDUCT RESEARCH IN VHEMBE AND MOPANI DISTRICT CLINICS AND HEALTH CARE CENTERS

Dear Sir / Madam

My name is Seemole Cedrick Hlako, and I am an MSc Public Nutrition student at the University of Venda (UNIVEN). The research I wish to conduct for my Master's dissertation is titled "Iodine Status of Lactating Women and Infants aged 0 to 6 months in Mopani and Vhembe District, Limpopo Province". This project will be conducted under the supervision of Dr L.F Mushaphi (Head of Department of Nutrition and Senior Lecturer UNIVEN) and Mr NS Mabapa (Senior Lecturer, Department of Nutrition, UNIVEN).

I am hereby seeking your consent to approach a number of Clinics and Health Centers in the Vhembe and Mopani district to provide participants for this project. I have provided you with a copy of my Research Proposal which includes copies of the measure, consent and assent forms to be used in the research process, as well as a copy of the approval letter which I received from the UNIVEN Research Ethics Committee (Human).

Upon completion of the study, I undertake to provide the department of Health Limpopo with a bound copy of the full research report. If you require any further information, please do not hesitate to contact me on 015 962 8683, cell 072 3411354, **and cedrickhlakoo@.gmail.com Thank you for your time and consideration in this matter.**

Yours Sincerely

Cedrick Hlako

APPENDIX B: RECRUITMENT LETTER

TO: The Parent/Legal guardian

RESEARCH PROJECT: Cross-sectional study to determine iodine status of lactating mothers and infants aged 0 to 6 months in Mopani and Vhembe District of the Limpopo Province, South Africa

Researchers
Mr Seemole Hlako Msc Public Nutrition student, University of Venda.
Dr Lindelani Mushaphi PhD, RD (SA) School of Health sciences, Department of Nutrition University of Venda.
Mr Solomon Mabapa Msc Public Nutrition, Lecturer, University of Venda.

Dear sir/madam

We would like to invite you and your child to take part in a study. The aim of the study is to determine the iodine status of lactating women and their infants. Iodine is a mineral that is very much essential for a child to grow and learn very well.

If you are interested to take part in the study, one of our fieldworkers will make an appointment to meet with you and your child at your local clinic and at your house.

What will be expected from you and your child in the study?

- You will be asked questions about your socio-demographic information and iodine.
- You will be expected to give your sample of breast milk, urine sample, household salt sample and as well as household water sample.
- Your infant will be expected to give a sample of his/her urine.

You and your child will not benefit directly, but the information we get from you and your child will help us to determine the levels of your breast milk iodine concentrations and you and the child s iodine status. This information can help the Department of Health to understand your iodine status and that of your child, so that they can make proper and informed decisions

Contact Numbers...

Address...

APPENDIX C: CONSENT FORM

TO: Parent/Legal guardian and Infant

RESEARCH PROJECT: Cross-sectional study to determine iodine status of lactating mothers and infants aged 0 to 6 months in Mopani and Vhembe District of the Limpopo Province, South Africa

Researchers

Mr Seemole Hlako Msc Public Nutrition student, University of Venda.

Dr Lindelani Mushaphi PhD, RD (SA) School of Health sciences, Department of Nutrition, University of Venda.

Mr Solomon Mabapa Msc Public Nutrition, Lecturer, University of Venda.

The aim of this study is to determine the iodine status of lactating mothers and infants in Mopani and Vhembe districts of the Limpopo Province, South Africa. Lactating mothers are required to provide their breast milk and urine for determination of iodine status. Water and salt samples will also be required to determine iodine content. Information on demographic and factors influencing iodine status will be collected by means of a questionnaire. Urine of infants will be required. The information provided in this study will be kept confidential that means names will not be recorded but codes will be used instead. If you agree to participate in the study, please sign below.

I understand that:

1. The study deals with the prevalence of iodine status of lactating mothers and infants in Vhembe and Mopani district of the Limpopo Province, South Africa.
2. Any question that I may have regarding the research or related matters will be answered by the researcher.
3. The researcher will require breast milk, urine, salt and water samples for determination of iodine content. Questionnaire will be used for demographic information and factors influencing iodine status.
4. A professional nurse will clinically assess you and your infant
5. The research protocol, i.e. the aim, objectives and methods have been explained to me.
6. Participation in this study is by choice, and I may withdraw my participation at any stage without any action taken against me.

I.. agree to participate in the study

Signature.. Date...

Signature.. Date..

(Researcher)

APPENDIX D: QUESTINNAIRE

Iodine status of lactating mothers and Infants aged 0 to 6 months in Mopani and Vhembe District of the Limpopo Province, South Africa.

DATE OF INTERVIEW: ....

CODE

SECTION A DEMOGRAPHIC INFORMATION

1. Age of the mother

< 18	1.
18 – 24	2.
25 – 35	3.
36 – 45	4.
> 45	5.

2. Age of the infant.................

1. Less than one Month	1.
2 Months	2.
3 months	3.
4 Months	4.
5 Months	5
6 Months	6

3. Gender of the infant

Boy	Girl
1.	2.

3. Are you currently employed?

Health Professional	1.

Retail Industry	2.
Security	3.
Cleaning services	4.
Education	5.
Other	6.
Unemployed	7.

4. Household income per month

> 500	1.
500 – 1000	2.
1001 – 3500	3.
3501-6000	4.
> 6000	5.

5. Level of education

Grade 1-7	1.
Grade 8-10	2.
Grade 11-12	3.
Tertiary Education	4.
None	5.

6. Marital Status

Single	Married	Widowed	Divorced	Living with a partner
1.	2.	3.	4.	5.

SECTION B FACTORS AFFECTING IODINE STATUS OF LACTATING WOMEN AND INFANTS

7. Are you exclusively breastfeeding (If less than 6 months old)

Yes	No
1.	2.

8. When do you add salt?

1.While eating	1.
1.During cooking	2.
Before eating	3.
Do not add salt	4.

9. Do you know about salt iodization/fortification?

YES	NO
1.	2.

10. Which salt do you use?

1. Fine salt	1.
2. Coarse salt	2.
3. Both	3.
4. None	4.

11. How often do you eat cabbage?

1. Daily	1.
2. Once in a week	2.
3. Once in a month	3.
4. Twice per month	4.
5. Never	5.

11. How do you eat your cabbage?

1. Cooked	1.
2. Uncooked	2.
3. Both	3.
4. Never	4.

12. How often do you eat sweet potato?

1.Daily	1.
2.Once in a week	2.
3.Once in a month	3.
4.Twice per month	4.
5.Occasionally	5.
6. Never	6.

13. Where do you usually buy or obtain salt that is used for food in your house?

Buy in Supermarkets such as pick and pay, Spar, Shoprite etc	1.
Agricultural coarse salt from pension pay points	2.
Spaza Shop	3.
Street vendor or hawker	4.
Either combination	5.

14. Do you read nutrition information on food items?

Yes	No
1.	2.
Cannot read	3.

15. Do you check salt content of food before buying?

Yes	No
1.	2.

16. Can you identify the formula for salt on food packages?

Yes	No
1.	2.

17. Identify salt formula on this food package

Yes	No
1.	2.

18. Do you have any concern about iodine being added to salt?

Yes	No

1.	2.
Unsure	3.

SECTION C

19. **SAMPLE COLLECTION**

SAMPLE	**COLLECTED**	**NOT COLLECTED**
Breast Milk	Yes	No
Infant Urine	Yes	No
Mother urine	Yes	No
Household water	Yes	No
Salt sample	Yes	No

APPENDIX E: ETHICAL CERTIFICATE

RESEARCH AND INNOVATION
OFFICE OF THE DIRECTOR

NAME OF RESEARCHER/INVESTIGATOR:
Mr SC Hlako

Student No:
11618373

PROJECT TITLE: **Iodine status of lactating women and infants aged 0 to 6 months in Mopani and Vhembe District of the Limpopo province, South Africa.**

PROJECT NO: **SHS/17/NUT/05/1108**

SUPERVISORS/ CO-RESEARCHERS/ CO-INVESTIGATORS

NAME	INSTITUTION & DEPARTMENT	ROLE
Dr LF Mushaphi	University of Venda	Supervisor
Mr NS Mabapa	University of Venda	Co- Supervisor
Mr SC Hlako	University of Venda	Investigator – Student

ISSUED BY:
UNIVERSITY OF VENDA, RESEARCH ETHICS COMMITTEE

Date Considered: August 2017
Decision by Ethical Clearance Committee Granted
Signature of Chairperson of the Committee: ....
Name of the Chairperson of the Committee: Prof. G.E. Ekosse

UNIVERSITY OF VENDA
DIRECTOR
RESEARCH AND INNOVATION
2017 -08- 22
Private Bag X5050
Thohoyandou 0950

University of Venda
PRIVATE BAG X5050, THOHOYANDOU, 0950, LIMPOPO PROVINCE, SOUTH AFRICA
TELEPHONE (015) 962 8504/8313 **FAX** (015) 962 9060
"A quality driven financially sustainable, rural-based Comprehensive University"

APPENDIX F: UHDC APPROVAL LETTER

UNIVERSITY OF VENDA

OFFICE OF THE DEPUTY VICE-CHANCELLOR: ACADEMIC

TO :	MR/MS S.C HLAKO
	SCHOOL OF HEALTH SCIENCES
FROM:	PROF J.E. CRAFFORD
	DEPUTY VICE-CHANCELLOR: ACADEMIC
DATE :	04 JULY 2017

DECISIONS TAKEN BY UHDC OF 13TH JUNE 2017

Application for approval of Master's research proposal in Health Sciences: **S.C Hlako (11618373)**

Topic: "Iodine Status lactating women and infants aged 0 to 6 months in Mopani and Vhembe District of the Limpopo Province, South Africa."

Supervisor	UNIVEN	Dr. L.F Mushaphi
Co-supervisor	UNIVEN	Mr. N.S Mabapa

UHDC approved Master's proposal

Prof J.E. CRAFFORD
DEPUTY VICE-CHANCELLOR: ACADEMIC

APPENDIX G: PROVINCIAL DEPARTMENT OF HEALTH PERMISSION LETTER

LIMPOPO
PROVINCIAL GOVERNMENT
REPUBLIC OF SOUTH AFRICA

DEPARTMENT OF HEALTH

Enquiries: Stols M.L (015 293 6169) Ref:4/2/2

Hlako C
Department of Nutrition
University of Venda
Private Bag X5050
Thohoyandou
0950

Greetings,

RE: Iodine Status of Lactating Woman and Infants aged 0-6 months in Mopani and Vhembe Districts, Limpopo Province

The above matter refers.

1. Permission to conduct the above mentioned study is hereby granted.
2. Kindly be informed that:-
   - Research must be loaded on the NHRD site (http://nhrd.hst.org.za) by the researcher.
   - Further arrangement should be made with the targeted institutions, after consultation with the District Executive Manager.
   - In the course of your study there should be no action that disrupts the services.
   - After completion of the study, it is mandatory that the findings should be submitted to the Department to serve as a resource.
   - The researcher should be prepared to assist in the interpretation and implementation of the study recommendation where possible.
   - The above approval is valid for a 3 year period.
   - If the proposal has been amended, a new approval should be sought from the Department of Health.
   - Kindly note, that the Department can withdraw the approval at any time.

Your cooperation will be highly appreciated.

Head of Department Date 12/09/2017

18 College Street, Polokwane, 0700, Private Bag x9302, POLOLKWANE, 0700
Tel: (015) 293 6000, Fax: (015) 293 6211/20 Website: http/www.limpopo.gov.za

APPENDIX H: VHEMBE DISTRICT APPROVAL LETTER

LIMPOPO
PROVINCIAL GOVERNMENT
REPUBLIC OF SOUTH AFRICA

DEPARTMENT OFHEALTH
VHEMBE DISTRICT

Ref: S5/6
Enq: Muvari MME
Date: 21 November 2017

Dear Sir/ Madam ; Hlako Cedrick

REQUEST FOR PERMISSION TO CONDUCT RESEARCH IN VHEMBE DISTRICT CLINICS AND HEALTH CENTRES

1. The above matter bears reference
2. Your letter received on the 21/11/2017 requesting for permission to access Vhembe institutions for research is hereby acknowledged
3. The District has no objection to your request.
4. Permission is therefore granted for the request to access Vhembe District institutions
5. You are however advised to make the necessary arrangements with the facilities concerned.
6. Wishing you success in your research in Vhembe health facilities .

.... 22/11/2017..........
DISTRICT CHIEF DIRECTOR DATE

Private Bag X5009 THOHOYANDOU 0950
OLD parliamentary Building Tel (015) 962 1000 (Health) (015) 962 4958 (Social Dev) Fax (015) 962 2274/4623
Old Parliamentary Building Tel: (015) 962 1848, (015) 962 1852, (015) 962 1754, (015) 962 1001/2/3/4/5/6 Fax (015) 962 2373, (015) 962 227

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APPENDIX I: MOPANI DISTRICT APPROVAL LETTER

LIMPOPO
PROVINCIAL GOVERNMENT
REPUBLIC OF SOUTH AFRICA

DEPARTMENT OF HEALTH
MOPANI DISTRICT

Ref: S4/2/2
Enq: Mohatli IE
Tel: 015 811 6543

To **Hlako C**
Department of Nutrition
University of Venda
Private Bag X5050
Thohoyandou
0950

Re: PERMISSION TO CONDUCT RESEARCH IN MOPANI HEALTH FACILITIES: YOURSELF

1. The matter cited above bears reference
2. This serves to respond to the request submitted to research on the topic: **"Iodine Status of Lactating Woman and Infants aged 0-6 months in Mopani District, Limpopo Province."**
3. It is with pleasure to inform you about the decision to permit you to conduct research in the facilities within Mopani District.
4. You will be required to furnish the hospital and PHC authorities with this letter for purposes of access and assistance.
5. You are further advised to observe ethical standards necessary to keep the integrity of the facilities.
6. The Mopani District wishes you well in your endeavour to generate knowledge.

Director: Corporate Services
Date: 2018/02/19