LEVELS OF NITRATES, NITRITES AND SELECTED TOXIC METALS IN BRANDS OF INFANT FORMULA MILK FROM NAIROBI COUNTY, KENYA

BY

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APRIL, 2014
DECLARATION

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

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Signature------------------------ Date----------------------

We confirm that the work reported in this thesis was carried out by the candidate under our supervision as University Supervisors.

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Signature------------------------ Date----------------------
DEDICATION

Special dedication to my father, the late

Joel Elkana Odhiambo
ACKNOWLEDGEMENT

My most sincere gratitude to God the Almighty for availing to me the means and ability to pursue this course.

I wish to express my sincere gratitude to my supervisors Dr. Wanjau Ruth and Dr. Nawiri Mildred for their unwavering support and guidance from the inception to the completion of this research work. I’m grateful to them for sharing with me their expertise knowledge and immense experience in the research and for their inspiration.

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# Abbreviations and Acronyms

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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AAS</td>
<td>Atomic Absorption Spectrometer</td>
</tr>
<tr>
<td>ADD</td>
<td>Attention Deficit Disorder</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>CDC</td>
<td>Center for Disease Control</td>
</tr>
<tr>
<td>EWI</td>
<td>Estimated Weekly Intake</td>
</tr>
<tr>
<td>FAO</td>
<td>Food and Agricultural Organization</td>
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</table>
ABSTRACT

Infant formula milk has been increasingly used as a breast milk substitute as a result of maternal occupation, death and illness. This happens despite the United Nations, World Health Organization (WHO), international and national health agencies recommending exclusive breast feeding during the first six months of infancy. Following this, infant formula milk is increasingly being associated with infant health complications and even infant deaths due to its contamination during processing and contamination of its raw materials. In 2008, contamination of infant formula milk with melanine in China lead to serious illness in more than 300,000 children and death of at least six babies. Exposure of infants to high levels of nitrates and nitrites can lead to methemoglobinemia while metals such as aluminium, cadmium, lead, nickel, and zinc have adverse effects to the kidney, brain development as well as causing nausea and loss of appetite to an infant. The WHO and Food and Agricultural Organisation (FAO) have set maximum levels of concentration of these ions and metals in infant formula milk and levels of provisional tolerable weekly intake (PTWI) of these ions and metals by infants. The Kenya Bureau of Standards
(KEBS) too has set the maximum level of concentration of Pb in infant formula milk. There is no information on the levels of these nitrates, nitrites and metals in infant formula milk marketed in Nairobi County, Kenya. This study determined concentration of \( \text{NO}_3^-, \text{NO}_2^- \), Al, Cd, Pb, Ni and Zn, in seven brands of purposively selected infant formula milk sold in Nairobi County for feeding infants aged 0-6 months. The techniques used were Ion selective electrode (ISE) and atomic absorption spectroscopy (AAS) while analysis of variance (ANOVA) was used for data analysis. Except for \( \text{NO}_2^- \) and Cd, \( \text{NO}_3^- \), Al, Pb, Ni and Zn were detected in all the brands of infant formula milk. The mean (n=9) levels (µg/mg) of the nitrate and metals ranged as follows; \( \text{NO}_3^- \): 0.022±0.004 (brand code U) – 0.035±0.004 (brand code Z); Al: 1.054±0.085 (brand code T) – 2.156±0.423 (brand code X); Pb: 0.018±0.002 (brand code T) – 0.059±0.002 (brand code W); Ni: 0.022±0.001 (brand code X) – 0.032±0.002 (brand code V) and Zn: 24.00±0.52 (brand code T) – 32.04±0.74 (brand code U). There was significant difference in the levels of the ions and metals in the different brands of infant formula milk. The levels were found to be below the limits set by WHO/FAO. However, the concentration of Pb was found to be higher than the limits set by KEBS in all the brands except brand codes T and V. The levels of Zn were below those indicated in the labels of infant formula milk. The estimated weekly intake (EWI) of the ions and metals was found to be below Provisional Tolerable Weekly Intake (PTWI) showing safety of the infant formula milk. However, there is a risk of bioaccumulation.
CHAPTER ONE: INTRODUCTION

1.1 Background Information

The World Health Organization (WHO), international and national health agencies recommend exclusive breastfeeding for the first six months of infancy (CDC, 2006). However, a baby’s health may depend on safe and nutritious alternatives other than breastfeeding in cases such as death of the mother, risk of mother to child transmission of human immunodeficiency virus and other infectious diseases such as tuberculosis, absence of the mother from the infant for an extended period of time due to work, study or other engagements, personal preferences, personal beliefs and societal pressure (Lawrence, 2004; WHO, 2004; Mamiro et al., 2005; CDC, 2006). In Kenya, it is reported that only about 32% of lactating mothers exclusively breastfeed their babies during their first six months of infancy (Kenya National Bureau of Statistics, 2010). This leads to use of breast feeding substitutes such as dairy milk, sweetened liquids and infant formula milk. Beside breast milk, infant formula milk is the only other product which is considered nutritionally acceptable for infants under the age of one year (WHA, 2001). Infant formula milk has a special role to play in the diet of infants as a major source of energy and nutrients for babies who are not being breastfed (Gian et al., 2009). In Nairobi 18.1% of the infants who are not exclusively breastfed use infant formula milk (Muchina and Waithaka, 2010).

Infant formula milk prepared in accordance with the standards of Codex Alimentarius Commission is considered as a safe complementary food and suitable breast milk substitute (WHA, 2001). However, metal pollution as a result of increasing
industrialization throughout the world has penetrated into all sectors of the food industry including infant formula milk (Gian et al., 2009). Furthermore many elements and ions can be present in foods naturally, or through human activities, such as processing, packaging, farming activities and industrial emission (Khalifa and Ahmad, 2010). Zinc is important for growth and muscle development of an infant and plays an important role in cell division which is massive during infancy, thus it is intentionally added to infant formula milk. This addition may result in levels that cause toxicity (Heird, 1999). Nitrate and nitrites ions occur naturally in the atmosphere as part of nitrogen cycle and therefore are found in the raw material for processing infant formula milk. Nitrates are also used as food preservatives (WHO, 2006).

Studies have reported contamination of infant formula milk by various substances such as nitrates, nitrites, aluminium, cadmium, mercury, nickel, lead and melamine (Reinik, 2005; Gian et al., 2009; Khalifa and Ahmad, 2010; Burrell and Exley, 2010; Ljung et al., 2011). Water that is used to reconstitute the infant formula milk can also be a source of contaminants such as lead and nitrate ion (Ljung et al., 2011). In 2008 melamine contamination of infant formula milk in China lead to deaths and illness to several infants. Other cases of recall of infant formula milk in developed world as a result of deficiency in necessary nutrients and contamination have also been reported (Marsha, 2010). In 2006 Mead Johnson Company recalled a batch of its infant formula milk due to contamination with metal particles (Nakashima et al., 2009). A study to determine the concentration of selected trace and toxic elements in breast milk and infant formula milk reported the concentrations in infant formula milk to be tenfold higher than in breast
milk, thus confirming infant formula milk as an exposure route of toxic elements to infants (NDRC, 2005).

Infants are at a critical point of their brain development and exposure to elements such as lead and cadmium pose severe health risk to the child and can lead to speech delay, hyperactivity, learning disabilities, attention deficit disorder and neurological deficits (Nevin, 2000; Needleman et al., 2002; Lanphear et al., 2008). The effect of metal poisoning on infants is compounded by the fact that even at low levels of exposure metals bioaccumulate in vital organs such as the kidney, an effect that persists in adulthood (Mielke et al., 1999). The relationship between nitrate, nitrite ions and infant methemoglobinemia, nausea and diarrhea has been well reviewed and appreciated, nitrates and nitrites may also cause endogenous formation of carcinogenic N-nitroso compounds (Meah et al., 2004). Nitrite is reported to arise from microbiological reduction of nitrate in foods when they are stored at room temperature (Ezeagu, 1996). The problem is particularly acute in infants since they are more sensitive to the effects of nitrite (Lutynski et al., 1996). Chemical composition of infant formula milk also depends on the duration of its storage and therefore should be used as early as possible from the date of manufacture (Salah, 2012). The WHO/FAO have set in place provisional tolerable weekly intake limits for these ions and metals by infants (Ljung et al., 2011).

There are more than seven brands of infant formula milk marketed in Nairobi including but not limited to Nan, Lactogen and Infacare. These brands are imported from countries such as Netherlands, Ireland, Poland, South Africa and United Arabs Emirates. The infant
formula milk is marketed for use by infants in different age brackets of 0-6 months, 7-12 months and beyond one year. Kenya is a signatory to all global conventions that commit to promote, protect and support infant and young children feeding practices (KEBS, 2006; Komen, 2009). In September 2012 parliament passed the breast milk substitutes Regulation and Control Bill so as to provide for appropriate marketing and distribution of safe and adequate nutrition for infants (The Sunday Standard, 2012). Monitoring levels of ions and metals in infant formula milk is of considerable health importance because of the known toxicity to infants yet there is no information on the levels of NO$_3^-$, NO$_2^-$, Al, Cd, Pb, and Ni in infant formula milk marketed in Nairobi. Furthermore the labels of various brands of infant formula milk do not indicate whether these ions and metals are present. Based on the feeding tables provided by the manufacturers it is possible to calculate the estimated weekly intake of these ions and metals by the infants and compare the same with Provisional Tolerable Weekly Intake provided by WHO/FAO. There is need therefore to determine the levels of these ions and metals in infant formula milk sold in Nairobi County to ascertain their safety.

1.2 Problem statement and justification

More than 70% of lactating mothers in Kenya do not breast feed their infants exclusively for the recommended periods of six months (KNBS, 2010). As a result infants are subjected to intake of formula milk. The US Foods and Drugs Administration in 2002 stated that infant formula milk was not a sterile product (Lanphear et al., 2008). Its use poses risks of exposure to infants to toxic metals and ions. Moreover, labels on the packaging of the infant formula milk do not exhibit the levels of the ions and metals.
Despite the standards set for marketing the products it is important that the levels of NO$_3^-$, NO$_2^-$, Al, Cd, Pb, Ni and Zn in infant formula milk sold in Nairobi County be determined.

1.3 Hypotheses
There is no significant difference in the levels of NO$_3^-$, NO$_2^-$, Al, Cd, Pb, Ni and Zn in formula milk for infants aged 0-6 months in Nairobi County.

1.4 Objectives
1.4.1 General objective
To determine the concentration of nitrates, nitrites and selected toxic metals in selected brands of infant formula milk sold in Nairobi County, Kenya.

1.4.2 Specific objectives
(i) To determine the concentration of NO$_3^-$ and NO$_2^-$ in selected brands of formula milk for infants aged 0-6 months sold in Nairobi County.
(ii) To determine the concentration of Al, Cd, Pb, Ni and Zn in selected brands of formula milk for infants aged 0-6 months sold in Nairobi County.

1.5 Significance of the study
While it would be assumed that infant formula milk is approved by KEBS prior to delivery to the market, the quantities of NO$_3^-$, Al, Pb and Ni raise concerns to address the labels and further sensitize the public on their use. It is known that infants who are fed on infant formula milk consume more than is advised in the feeding tables. This further
enhances exposure to the ions and elements. Bioaccumulation of toxic elements can result into adverse health effects in infants.

1.6 Scope and limitations

Only seven brands of formula milk marketed in Nairobi for infants aged 0-6 months were studied. This study analyzed for NO$_3^-$, NO$_2^-$ and the metals Al, Cd, Pb, Ni and Zn. The sources of the infant formula milk were not considered as these are imported products. Breast milk was not studied but comparisons were made with standards set by WHO/FAO and KEBS. This study was based on the brand names even though some manufacturers have more than one brand in the market. Though water used to reconstitute the infant formula milk can be a source of contaminants, deionized water will be used in this study. The infant formula milk sampled were those that had stayed for less than one year from the date of manufacture.
CHAPTER TWO: LITERATURE REVIEW

2.1 Contaminants in infant formula milk

According to the US Federal Food, Drugs and Cosmetic Act, infant formula milk is food which purports to be or is represented for special dietary use solely as food for infants by reasons of its simulation of human milk or its suitability as a complete or partial substitute for infant milk (Wells, 1996). It is usually in powder form and is prepared for bottle-feeding or cup-feeding after dilution with water. The most commonly used infant formula milk contain purified cow’s milk as a source of protein, blend of vegetable oils as a fat source, lactose as a carbohydrates source, vitamin-mineral mix and other ingredients depending on the manufacturer (Ryan, 1997). There has been a significant increase in the production and sale of infant formula milk in the world with the global market estimated at US $7-9 billion by 2010 (Robert, 2004). Many of the major companies manufacturing infant formula milk are based in America, Asia and Europe; these companies market their products all over the world including Kenya.

According to WHA (2001) infant formula milk prepared according to the Codex Alimentarius Standards is a safe complementary food and a suitable substitute to breast milk. However, infant formula milk has since been increasingly associated with serious infant illness as a result of contamination with various substances (WHO, 2003). A number of studies have reported contamination of infant formula milk by \( \text{NO}_3^- \), \( \text{NO}_2^- \) and various elements. Kerr (2011) reported China’s health authority calling for stricter limits of metals in infant food after scientists voiced concerns over the risk of toxic elements such as arsenic, cadmium and lead in infant food.
In 2008, there was massive contamination of infant milk with melamine in China. This led to serious illness in more than 300,000 children including cases of acute kidney failure and death of at least six babies (Kerr, 2011). In November 2008, traces of melamine were reported to have been found by the Food and Drugs Administration in infant formula milk sold in the US and manufactured by the three major firms (Kerr, 2011). A World Health Assembly meeting in 2005 following cases of contamination of infant formula milk with Enterobacter sakazakii passed a resolution that urged member states to ensure health care personnel, parents and care givers are informed that infant formula milk may contain pathogenic microorganisms.

Despite drinking water being a major exposure route of infants to nitrate and nitrite ions, industrial products such as infant formula milk are also a source of nitrates and nitrites to infants (Meah et al., 2004). Though nitrate ion has a low level of acute toxicity to infants, it may constitute a health problem if it is reduced to a nitrite ion. Reduction to nitrite ion may take place in presence of bacteria or in contact with metals (Chou et al., 2003). Reinik (2005) reported that the concentration of nitrate ion in infant formula milk in Estonia was 2 mg/Kg, this was below the set limit but still was a source of concern as infants are susceptible to health effects of nitrates. Erkekoglu and Baydar (2009) reported nitrite contamination in infant formula in Turkey as 0.204 µg/mg, this was below the recommended limits. They concluded that the contamination arose from several sources during the manufacturing process and called for care during manufacturing to reduce the concentration of nitrite.
Tasneem et al. (2009) in a study to determine the levels of toxic elements in different brands of infant formula milk in Pakistan detected Al, Cd and Pb in the range of 1070-2170, 10.5-34.4 and 28.7-119 µg/kg respectively. They observed that though the estimated intakes of these toxic elements were below the minimum allowed levels, their presence in infant food was a source of concern and regular checks needed to be conducted on the infant products. In 2006, the joint FAO/WHO Expert Committee on Food Additives re-evaluated the safety of aluminium and lowered its Provisional Tolerable Weekly Intake (PTWI) by seven fold to 1 mg/kg of body weight due to its toxicological effects in infants (Ferreira et al., 2008). Khalifa and Ahmad (2010) analyzed various brands of infant formula milk sold in Riyadh Saudi Arabia for levels of various toxic and non toxic elements. They reported the concentration of Al, Cd, Pb and Zn in parts per million as 1.944 ±1.09, 0.007 ± 0.005, 0.018 ± 0.002 and 35.7 ± 0.853 respectively. They observed that though these concentrations were below the WHO guidelines, their presence in infant formula milk was of great concern since infants are very sensitive to the toxic effects of these elements. Burrell and Exley (2010) reported the concentration of aluminium in some infant formula milk in United Kingdom to be forty times higher than the concentration in breast milk, they called on the manufacturers of infant formula milk to urgently reduce the concentration of aluminium to as low levels as possible.

In spite of comparatively rigorous system of manufacture in the US, several cases of recall of infant formula milk have occurred as a result of contamination by toxic
substances such as metals and bacteria, deficiency of necessary nutrients and incorrect labeling (Friedman, 2010). In 2010, a major infant formula milk company recalled over 100 million units of its products due to contamination with beetle parts and beetle larvae; another company that manufactured infant formula milk for use by preterm babies recalled its products in 2007 because the milk was iron deficient (Marsha, 2010). In 2012, a major dairy firm in China recalled some of its infant formula products after the country’s product quality watchdog reported mercury concentrations of 0.034 – 0.045 mg/Kg (BBC, 2012). The US Food and Drugs Administration in 2002 stated that infant formula milk was not a sterile product hence the need to monitor regularly the infant formula milk sold in the market (Lanphear et al., 2008). In 1985, Syntex an infant formula milk firm in US was ordered to pay $27 million compensation for the deaths of two infants who suffered brain damage after drinking the company’s infant formula milk (Mount, 1985).

The Natural Resources Defense Council (2005) in Germany determined the concentration of numerous essential and toxic elements in human milk and selected infant formula. Most of the concentrations in the infant formula were approximately tenfold higher than in the human milk as shown in Table 2.1
Table 2.1: Comparison of the concentration of various elements in breast and selected infant formula milk in Germany

<table>
<thead>
<tr>
<th>Element</th>
<th>Concentration (µg/dL)</th>
<th>Breast milk</th>
<th>Infant formula milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cobalt</td>
<td>0.2</td>
<td>0.2</td>
<td>0.9</td>
</tr>
<tr>
<td>Chromium</td>
<td>24.3</td>
<td>24.3</td>
<td>6.9</td>
</tr>
<tr>
<td>Iron</td>
<td>380</td>
<td>380</td>
<td>9227</td>
</tr>
<tr>
<td>Manganese</td>
<td>6.3</td>
<td>6.3</td>
<td>46.1</td>
</tr>
<tr>
<td>Nickel</td>
<td>0.8</td>
<td>0.8</td>
<td>14.3</td>
</tr>
<tr>
<td>Selenium</td>
<td>17</td>
<td>17</td>
<td>Not quantified</td>
</tr>
<tr>
<td>Vanadium</td>
<td>0.2</td>
<td>0.2</td>
<td>0.7</td>
</tr>
<tr>
<td>Aluminium</td>
<td>67</td>
<td>67</td>
<td>210.5</td>
</tr>
</tbody>
</table>

Source: NDRC, 2005

In Kenya, there are no reports of study of contaminants in infant formula milk and it is of concern that packaging labels of these products do not display the concentrations of the ions and elements under investigation with the exception of zinc.

2.1.1 Nitrates and nitrites

Nitrate (NO\text{3}^{-}) and nitrite (NO\text{2}^{-}) are inorganic ions that occur naturally in the atmosphere and are part of the nitrogen cycle. Some of the most common nitrate compounds include sodium nitrate, potassium nitrate, ammonium nitrate and calcium nitrate. Nitrates are mainly produced for use as fertilizers due to their high solubility and biodegradability. Nitrates are used as food preservatives (WHO, 2006). Nitrite ion is reported to arise from microbiological reduction of nitrates in food and drinking water at room temperature.

The main exposure route of nitrates and nitrites to infants is through drinking water and preserved foods such as infant formula milk (Dusdieker, 1994). Exposure of infants to
nitrates and nitrites can lead to nausea, diarrhea and vomiting. At acute levels nitrite ion oxidize Fe$^{2+}$ in haemoglobin to Fe$^{3+}$ rendering it unable to carry oxygen resulting into lack of oxygen in the body tissue, a condition known as methemoglobinemia or the blue baby syndrome (Reinik, 2005). Reduced oxygenation of the body tissues can have adverse health implications to the infant including death. Infants of 0-3 months are at highest risk of methemoglobinemia because their normal intestinal flora contribute to the generation of methemoglobinemia (Pinar and Terken, 2010). Nitrite ion may also react with secondary amine to form N-nitrosoamine which is suggested as a possible carcinogen (Tamme, 2006).

A study by Reinik (2005) on the levels of nitrate and nitrites in food in Estonia reported concentration of nitrate in infant milk to be about 2 µg/mg while nitrite was about 0.03 µg/mg. The author concluded that though these concentrations were below the recommended limits, there was need to regularly monitor the concentration of these ions in infant food. Tai et al. (2013) investigated the levels of nitrate and nitrite ion in milk and milk powder in Taiwan and reported that nitrite concentration in infant formula milk was below detection limit while nitrate concentration ranged from 0.3 – 83.2 ppm. They noted that even though some infant formula milk samples had nitrate concentrations beyond the recommended limit, the dietary intake by the children was below the allowed daily intake (ADI) suggested by WHO and thus the products surveyed did not cause food safety risk. The US Environmental Protection Agency safe standard’s for nitrates in baby food is 10 ppm while for nitrite is 1 ppm (Reinik, 2005). The Joint Committee on Food Additives of the Food and Agriculture Organization and the EU Scientific Committee for
food in 2005 established ADI of nitrate ion to be 3.7 mg/kg of body weight while that of nitrite as 0.06 mg/kg of body weight (Pinar and Terken, 2010).

2.1.2 Aluminium

Aluminium is a silvery white metal that exists abundantly in the earth crust making about 8% by weight of the earth’s solid surface (Rondeau et al., 2008). Aluminium is a non essential element to human beings (Azza and Ghada, 2011). It does not occur naturally in its elemental metallic state but occurs combined with other elements such as oxygen, silicon and fluorine. It is the most widely used non-ferrous metal due to its remarkably low density and ability to resist corrosion (Hetherington, 2007). Some of its uses include manufacture of automotive and aircraft parts, packaging materials such as cans and foils, water treatment cooking utensils, electrical transmission cables and construction of windows and doors (Ferreira et al., 2008).

Aluminium once absorbed into the body is distributed to most organs within the body with accumulation at high dose mainly in bones, brain, muscle and kidney. In infants the physiologies of their gastrointestinal tract, kidney and blood barrier are not fully developed and this may predispose them to aluminium toxicity (Ferreira et al., 2008; Kazi et al., 2009). Aluminium toxicity can lead to reduced skeletal mineralization observed in preterm infants and infants with growth retardation. At high concentrations Al can cause neurotoxicity (Pennington and Schoen, 1995; Banks and Kastin, 2008). Studies have shown that increased Al deposits in the brain can lead to risk of Alzheimer’s disease (Hawkes, 2006; Ferreira et al., 2008; Rondeau et al., 2008).
Burrell and Exley (2010) reported that concentration of aluminium in several infant formula milk brands in United Kingdom was 40 times higher than what is found in breast milk. They recommended the need to urgently reduce the Al levels in formula milk so as to protect the infants from aluminium toxicity. Khalifa and Ahmad (2010) in a study of levels of key elements in commercially available infant formula in Saudi Arabia reported mean concentrations of aluminium in the infant formula milk as $1.944 \pm 1.09 \, \mu g/g$. Salah (2012) studied the levels of toxic metals in infant formula milk in Egypt and reported Al concentrations as $1.350 \pm 0.076 \, \mu g/g$. These studies noted that though the estimated weekly intake of aluminium by the infants was below PTWI there was need to reduce the levels of Al in infant formula milk due to the vulnerability of infants to its exposure to. Azza and Ghada (2011) noted that Al contamination of infant formula milk resulted from contamination of the raw materials but increased notably during processing and packaging. They called for measures to be taken to reduce this contamination.

In 2006, the joint FAO/WHO Expert Committee on Food Additives re-evaluated the safety of aluminium and lowered its PTWI by seven fold to 1 mg/kg of body weight due to its toxicological effects (Ferreira et al., 2008).

### 2.1.3 Cadmium

Cadmium is a lustrous, silver-white, ductile and malleable metal. It occurs naturally in the earth crust in combination with other metals like zinc and copper. It is a byproduct of zinc, lead and copper extraction (Jarup, 1998). Cadmium is toxic even at trace
concentrations and is commonly found in industries (Bearer, 2000). It is used extensively in electroplating, manufacture of nickel-cadmium batteries and some phosphate fertilizers contain cadmium (Taylor, 1997). Plants absorb cadmium and get it into the food chain (Nabulo et al., 2006). Exposure to cadmium has been associated with anaemia, osteomalacia, cardiovascular diseases and kidney failure (Berglund et al., 2000). Cadmium toxicity targets the kidney and bone which are crucial calcium metabolizing sites thus leading to a decrease in the amount of calcium in blood (Lkesson, 2000; Gaw et al., 2006). Cadmium is first transported to the liver through blood, in the liver it forms complexes with proteins and these complexes accumulate in the kidney (Berglund et al., 2000).

Salah (2012) studied the levels of toxic metals in infant formula milk in Egypt and reported cadmium concentrations of $0.210 \pm 0.016$ µg/g. Behrooz et al. (2009) studied levels of selected metals in infant formula milk in Iran and reported concentrations of cadmium in infant formula milk as $0.359 \pm 0.215$ µg/g. Anna (2009) conducted an assessment of infant exposure to cadmium through infant formula milk in Lublin and reported cadmium concentration range of 0.0004-0.02 µg/g in different brands of infant formula milk. These studies observed that the intake levels of cadmium were below the recommended levels, however due to bioaccumulation of metals the presence of cadmium in infant food was of great concern and the products needed regular monitoring to ascertain infant safety. In 2001 the FAO/WHO JECFA set the PTWI for cadmium at 7 µg/kg of body weight for infants (Salah, 2012).
2.1.4 Lead

Lead is a soft, grey and lustrous metal with a high density and low melting point. It occurs naturally in the earth crust combined with other elements such as sulphur, zinc and copper. Lead is widely used in industries in the manufacture of batteries, ammunition, solder wires, pipes, roofing materials and x-ray shielding devices (Mielke et al., 1999; Gaw et al., 2006). Many uses of lead such as paint manufacture and manufacture of tetraethyl lead, a fuel additive have been discontinued due to dangers of lead poisoning (Hermberg 2000; Nakashima et al., 2009).

Lead has often been called the leading environmental threat to children due to its high toxicity and elevated quantities in the environment (Cambra et al., 1999). Dispersion of lead in the environment and its bioaccumulation in plants results to presence of lead in food chain and industrial products such as infant formula milk (Khalifa and Ahmad, 2010). Lead is rapidly absorbed into the blood stream and has adverse effects on the central nervous system, cardiovascular system and the immune system (Bergesson and Lynn, 2008). Exposure of infants to lead even at trace quantity can cause retardation in IQ, interference with growth and development of the infant, attention deficit disorder, speech delay, hyperactivity and lower class standing (Nevin, 2000; Karin and Terry, 2004; Lanphear et al., 2008).

Behrooz et al. (2009) studied levels of selected metals in infant formula milk in Iran and reported concentrations of lead in infant formula milk as 0.384 ± 0.222 µg/g. They noted that these high levels of lead in infant formula milk posed serious health effects to the infants that called for more vigorous quality control measures. Anna (2009) conducted an
assessment of infant exposure to lead through infant formula milk in Lublin and reported concentration range of 0.094-0.450 µg/g in different brands of infant formula milk while Khalifa and Ahmad (2010) in a study of levels of key elements in commercially available infant formula in Saudi Arabia reported mean concentrations of lead in the infant formula milk as 0.018 ± 0.002 µg/g. They observed that though the intake levels of lead through infant formula was below the set limits, presence of lead in infant formula posed great health risks to the infants. Ljung et al. (2011) studied the concentration of toxic elements in infant formula milk in Sweden and reported concentration of lead in breast milk as 0.5 ± 0.3 µg/L and 1.42 ± 0.13 µg/L in infant formula milk. They noted that formula fed infants had a higher intake of toxic elements than breast fed infants hence advocated for exclusive breast feeding for infants below 6 months.

The maximum limit of lead in infant formula milk in Kenya is 0.02 ppm (Kenya Bureau of Standards, 2006). In 2001 the FAO/WHO JECFA set the PTWI for lead at 25 µg/kg of body weight for infants (Khalifa and Ahmad, 2010).

2.1.5 Nickel

Nickel is a silvery-white, hard, malleable and ductile metal. It occurs naturally in the earth crust combined with elements such as iron, sulphur and oxygen (Reilly, 2002). It alloys readily with metals such as iron, copper chromium and zinc. Stainless steel is an alloy of nickel, iron and chromium and is used extensively during food processing hence resulting into contamination of food with nickel. Nickel compounds are also used for
nickel plating, manufacture of ceramics and batteries and in hydrogenation of fats (Davis, 2000).

In small quantities, nickel is essential. It may serve as a cofactor or structural component of specific metalloenzymes with a variety of physiologic functions. Nickel has been shown to facilitate ferric iron absorption or metabolism (Robert, 2004). Nickel has several adverse health effects when taken in large quantities such as decrease in infant weight, dizziness, allergic reactions and can lead to reduced lung function (Reilly, 2002). Nickel and certain nickel compounds have been listed by the National Toxicology Program (NTP) as being reasonably anticipated to be carcinogens (Ballatori, 2002).

A study by Food Standards Agency in United Kingdom in 2006 reported the mean concentration of nickel in various brands of infant formula milk as 0.1 mg/kg (Gaw et al., 2006) This exceeded the recommended limits thus posing a health risk to the infants. Michael et al. (2000) reported that the concentration of nickel in some infant formula milk sold in Austria was 17.9 µg/L, ten times more the concentration in breast milk while a study by NRDC in 2005 reported that nickel concentration in infant formula milk was 17 times more than in breast milk. This study demonstrated the health risk associated with infant formula milk. Abua et al. (2002) studied the concentration of various elements in infant formula milk in United States and reported concentration of nickel as 0.02 µg/L. They observed that this concentration was below the recommended limit but stated the need to regularly monitor nickel levels in infant formula milk. The acceptable limit for nickel is 0.07 mg/L of drinking water (WHO, 2005).
2.1.6 Zinc

Zinc is a hard, brittle, blue-white lustrous metal. It is the 24\textsuperscript{th} most abundant element in the earth crust. It occurs in the earth crust combined with other elements such as copper and lead. It readily forms alloys with other metals such as copper, aluminium and silver. Zinc metal is mostly used as an anti-corrosion agent through galvanization. It is also used as an anode material in the manufacture of batteries. Brass an alloy of zinc and copper is useful in the manufacture of communication equipment and musical instruments (Emsley, 2001).

Zinc is an important micronutrient for a child’s overall health and development (WHO, 2003). It plays an important role as an integral part of coenzymes of many enzymes including synthesis and metabolism of proteins and nucleic acids (Picciano, 2001). Zinc is a vital ingredient of infant formula milk with a RDA of 5 mg/day for infants (WHA, 2001). Intake of higher or lower amounts of zinc can affect the health of an infant (Milena \textit{et al}., 2009).

Zinc deficiency can occur due to insufficient intake or low bioavailability in the diet. Infants do not have zinc reserves at birth and thus depend entirely on their diet for it. Furthermore, the mineral is better absorbed when originating from breast milk than infant formula milk (Milena \textit{et al}., 2009). Zinc deficiency leads to an impairment of DNA, growth retardation and harms immune function. However, high concentration of zinc in infant formula milk may cause vomiting, renal damage (Doherty \textit{et al}., 2011).
A study by Milena et al. (2009) on the levels of essential elements in infant formula milk in Brazil reported concentration of zinc as 48.6 ± 0.1 µg/mg. Khalifa and Ahmad (2010) in a study of levels of key elements in commercially available infant formula in Saudi Arabia reported mean concentrations of zinc in the infant formula milk as 35.7 ± 0.833 µg/mg. These studies concluded that the concentrations were within the recommended limits. Ljung et al. (2011) studied concentration of various elements in infant formula milk in Sweden and reported concentration of zinc as 5-10 fold higher than in infant formula milk. The high concentration of zinc in infant formula posed health risks.

2.2 Methods of analysis

Analysis of nitrates and nitrites in milk and various food substances has been done using a number of techniques such as Flow-Injection Spectrophotometry (Reinik, 2005), High Performance Liquid Chromatography-Ultra Violet rays detection (HPLC-UV) (Chou et al., 2003) and Ion Selective Electrodes (ISE) (Rich et al., 2006).

Analysis of metals in matrices including infant formula milk has been done using a number of analytical techniques such as Energy Dispersive X-ray Fluorescence (EDXRF) (Beckhoff et al., 2006), Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Davidowski et al., 2010), Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) (Perkin, 2006), Differential Pulse Anodic Stripping Voltammetry (DPASV) (Behrooz et al., 2009), Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-
OES) (Abua et al., 2002), Instrumental Neutron Activation Analysis (INAA) (Paula et al., 2007) and Atomic Absorption Spectroscopy (AAS) (Gian et al., 2009).

The ISE has the advantages of reliability, wide concentration measurement range and ease of use while the AAS has the advantages of availability, high selectivity and sensitivity. These two methods were used for analysis of NO$_3^-$ and NO$_2^-$ ions and Al, Cd, Pb Ni and Zn respectively.

### 2.2.1 Principles of ion selective electrodes

An ISE is a transducer or a sensor that converts the activity of a specific ion dissolved in a solution into an electrical potential which can be measured by a voltmeter or a pH meter. The voltage develops in relation to an internal Ag/AgCl which acts as the reference electrode (Rich et al., 2006). It delivers real-time and on-line information on the presence and concentration of specific compounds or ions in complex (Buck and Lindner, 1994).

The ISE consists of a cylindrical tube made of a plastic material measuring 5-15 mm in diameter and 5-10 cm in length. An ion selective membrane is fixed at one end so that the external solution can come into contact only with the external surface. The other end is fixed to the reference electrode. The ion selective electrode and the reference electrode are connected by a milli-voltmeter. Measurement of concentration of the ion is done by immersing the electrodes in the test solution. Ion-selective membranes are currently available for a number of commonly occurring ionic species such as Br$^-$, CO$_3^{2-}$, Cl$^-$, CN$^-$, F$^-$, I$^-$, NO$_3^-$, NO$_2^-$, ClO$_4^-$, and SCN$^-$ (Khandpur, 2007).
An ion-selective membrane is the key component of all potentiometric ion sensors. It establishes the preference with which the sensor responds to the analyte in the presence of various interfering ions from the sample. If ions can penetrate the boundary between two phases, then an electrochemical equilibrium will be reached, in which different potentials in the two phases are formed. If only one type of an ion can be exchanged between the two phases, then the potential difference formed between the phases is governed only by the activities of this target ion in these phases. Activity coefficient of the ion being measured depends on the total ionic strength of the solution, any variation in concentration of ions present in the solution will affect the activity being measured. A total ionic strength adjustment buffer such solution is added to the test solution to maintain the total ionic strength (Khandpur, 2007). The ISE responds only to free ions and therefore presence of any species that complexes with ion being measured lower its activity. When the membrane separates two solutions of different ionic activities (\(a_1\) and \(a_2\)) and provided the membrane is only permeable to this single type of ion, the potential difference (E) across the membrane is described by the Nernst equation (Equation 2.1).

\[
E = \frac{RT}{zF} \ln \left( \frac{a_2}{a_1} \right)
\]

Equation 2.1

Where
- E = Potential difference
- R = Gas constant
- T = Absolute temperature
- F = Faradays constant
- Z = charge of ion
The potential difference can be measured between two identical reference electrodes placed in the two phases. In practice the potential difference is measured between an ion selective electrode and a reference electrode, placed in the sample solution. It is important to note that this is a measurement at zero current under equilibrium conditions. Equilibrium means that the transfer of ions from the membrane into solution is equal to the transfer from the solution to the membrane. The measured signal is the sum of different potentials generated at all solid-solid, solid-liquid and liquid-liquid interfaces.

Electrodes have two types of membranes:

a) **Solid Polymer Membrane**

This type of PCV membrane is used in the Nitrate, Calcium, and Ammonium ISEs. The membrane is a porous plastic disk, permeable to the ion exchanger but impermeable to the water. It allows the sensing cell to contact the sample solution and separates the internal filling solution from the sample. The PCV membrane electrodes have a replaceable membrane module.

b) **Solid State Membrane**

This is a thin crystalline membrane made from mono or polycrystallites of a single substance. The crystal separates the internal reference solution from the sample solution. They have good selectivity because only ions which can introduce themselves into the crystal structure can interfere with the electrode response. The voltage developed between the sensing and the reference electrodes is a measure of the concentration of the reactive ion being measured as the concentration of the ion electrodes.
2.2.2 Principles of atomic absorption spectroscopy

This is an analytical technique that involves generating a gaseous population of free atoms by heating a sample in a flame then passing a narrow bandwidth of light through the atoms. The atoms absorb light energy at certain wavelength and enter into an excited state. As the number of atoms in the light path increase, the amount of light absorbed also increase. By measuring the amount of light absorbed a quantitative determination of the amount of the analyte in the sample can be made. The use of special light sources and careful selection of wave lengths allows for specific determination of individual elements.

Absorption of light is associated with transition process from one steady state to another, for instance the case of a steady state O and J where $E_o < E_j$, the O-J transition results in the absorption of light with the frequency given in Equation 2.2.

$$
\nu_{oj} = \frac{E_j - E_o}{h}
$$

.................................Equation 2.2

Where
$h$ - Plank’s constant
$\nu$ - Frequency
$E_o$ - Energy at ground state
$E_j$ - Energy at the excited state
O - J – the transition stimulated by absorption of external radiation.

The number of atoms in the excited state relative to the number in the ground state is given by Maxwell –Boltzmann law (Skoog et al., 1998), given by Equation 2.3.
\[
\frac{N_1}{N_0} = \frac{g_1}{g_0} \exp \left( \frac{E_0 - E_1}{KT} \right) 
\]

.................................................................Equation 2.3

Where

- \(N_1\) - Number of atoms in the excited state
- \(N_0\) - Number of atoms in the ground state
- \(g_1\) and \(g_0\) – Statistical weight of excited and ground state respectively
- \(K\) - Boltzmann’s constant
- \(T\) - Absolute temperature
- \(E_0\) - Energy at the ground state
- \(E_1\) - Energy at excited state

The relative fraction of atoms in excited state is dependent on temperature whereas intensity is independent of temperature. Sample solution is aspirated through nebulizer into the air/acetylene or nitrous oxide/acetylene flame. An electrically heated graphite furnace is used when very high sensitivity is required. The sample solution gets dispersed into mist of droplets and then evaporated into dry salt. The dry salt goes into vapor and dissociates into atoms that absorb resonance radiation from external source. The unabsorbed radiation is allowed to pass through the monochromator which isolates spectral lines. The isolated analyte line falls on the detector and the output of which is amplified and recorded. The parameter measured is absorbance \((A)\) and related to concentration by the Equations 2.4 and 2.5.

\[
A = \log \left( \frac{I_0}{I} \right) 
\]

.................................................................Equation 2.4

\[
A = \varepsilon c l
\]

.................................................................Equation 2.5

Where;

- \(A\) - Absorbance
- \(I_0\) - Incidence radiation
- \(I\) - Attenuated radiation
- \(\varepsilon\) - Molar absorptivity \((\text{Lmol}^{-1}\text{cm}^{-1})\)
- \(c\) - Concentration \((\text{moldm}^{-3})\)
$l$ - Path length (cm)

Since the relationship between absorbance ($A$) and concentration ($c$) is linear over a wide range of levels (Beer’s law), standard solutions are used to obtain calibration curve. Levels of analyte in the sample are then established from the calibration curves.

A schematic diagram showing the major components of AAS is shown in Figure 2.1.

![Schematic diagram of AAS](image)

**Figure 2.1: Schematic diagram of AAS**
Source: Khandpur, 2007

(a) **Radiation sources**

There are two types of radiation sources namely

i) **Continuous source**

The continuous source gives a wide range of radiation and includes deuterium lamp and mercury vapor lamp. It is less sensitive because only a small amount of radiation passed by monochromator is absorbed while a large portion falls on the detector.

ii) **Hollow cathode source**
It is the commonly used radiation source in AAS. It is a discharge lamp which emits the characteristic light of the element to be analyzed. Hollow cathode lamp consists of a cylindrical thick walled glass tube which has a transparent window of glass affixed to one end. It contains an anode and a cup shaped cathode which is constructed of the element being analyzed. The glass tube is filled with neon or argon gas.

(b) Atomizer
The two types of atomizers are flame and electrothermal atomizers. In flame atomizer, the temperature is determined by flow rate and ratio of oxidant and fuel. In flame atomizer solvent is evaporated to produce solid molecular aerosol during dissolving process. Dissociation leads to atomic gas whereas some of the atoms ionize to give captions and electrons. In electrothermal atomizer, few molecules of the sample are first evaporated at low temperature and ashed at higher temperatures in electrically heated graphite. After ashing, the temperature is increased to 2000-3000 °C to cause atomization of the sample.

(c) Monochromators
They are filters, prisms or gratings that select the specific wavelength of light that is absorbed by the element in the sample. Diffraction gratings are preferred to prisms as they offer accuracy over a wide range of wavelengths and are cheaper. They isolate a single atomic resonance line from the spectrum of lights emitted by the hollow cathode lamp. This isolation allows for determination of specific element of interest when it is in presence of other elements. A monochromator comprises of an entrance slit, dispersion device and an exit slit. The entrance slit selects a defined beam of polychromatic light
from the hollow cathode source, the dispersion device causes the different wavelengths of light in the source beam to be dispersed at different angles and the exit slit enables a selection of a particular wavelength to produce the monochromatic light. Light selected by the monochromator is directed onto a detector.

(d) **Detectors**

A photomultiplier tube is the most commonly used detector in AAS. It gives a fast response and a linear response over a wide range of wavelengths. Detectors convert radiation energy into electrical signal. They measure wavelength of light transmitted and compares to the wavelength which originally passed through the sample. Other detectors include phototube and photodiode array detectors.

(e) **Read out system**

These are digital and interfaced with microprocessors that allow the programming of various aspects, bringing simplicity in operation.
CHAPTER THREE: MATERIALS AND METHODOLOGY

3.1 Research design

Experimental research design was used in this study. From one major store in Nairobi, formula milk for infants aged 0-6 months were purposively sampled. The brands were coded T-Z. Laboratory protocol procedures were followed for analysis of NO$_3^-$ and NO$_2^-$ using ISE and Al, Cd, Pb, Ni and Zn using AAS and results subjected to statistical test ANOVA prior to their presentation. Findings are discussed with reference to WHO/FAO and KEBS set standards for analysis of infant formula milk as well as compared with previous studies from other regions of the world. Further an estimated weekly intake of the ions and elements was calculated and compared to PTWI set by WHO/FAO.

3.2 Sampling

The seven brands of formula milk for infants aged 0-6 months in 500 g tin were purposively sampled from one major supermarket in Nairobi. Each brand of infant formula milk was sampled three times over a period of three months so as to obtain tins with different batch numbers. These brands were coded using letters T-Z.

Once in the laboratory the tins were opened and the formula milk in each tin mixed thoroughly to get a homogenous sample of each brand for digestion and analysis.

3.3 Cleaning of apparatus

All plastic and glassware apparatus used were washed with liquid detergent and rinsed with tap water. They were further be soaked in 10 % analytical grade nitric acid overnight and rinsed with de-ionized water and dried in an oven at 105 °C.
3.4 Chemicals and reagents
All chemicals and reagents used in this study were of analytical grade. Concentrated nitric acid, hydrochloric acid, hydrogen peroxide, sodium hydroxide pellets, potassium nitrate, sodium nitrite, aluminium sulphate, disodium phosphate, citric acid and silver sulphate were sourced from Thomas Backers Chemicals Ltd Mumbai India. Standards for zinc, nickel, cadmium and lead were purchased from Fluka Chemie GmbH Aldrich chemical company, inc. USA.

3.5 Preparation of standard stock solutions
For nitrate, potassium nitrate crystals of analytical grade were dried at 100 ºC overnight and 7.2 g accurately measured, dissolved in about 100ml distilled water then diluted to 1 litre to make 1000 ppm of solution. Ionic strength adjuster buffer solution for the analysis of nitrate ions was prepared by dissolving 6.6 g Al₂(SO₄)₃, 3.1 g Ag₂SO₄, 1.2 g H₃BO₄ and 1.9 g H₂NSO₄ in about 400 ml distilled water. Its pH was adjusted to 3.0 by slowly adding 0.1 M NaOH.

For nitrite ions, pure sodium nitrite was dried in an oven and 0.1 g of it accurately weighed. This was dissolved in 100 ml of distilled water in a 1L volumetric flask which was maintained in a water bath at 20 ºC for 30 minutes and distilled water slowly added to the mark. Ionic strength adjuster buffer solution for analysis of nitrite ion was prepared by dissolving 14.3 g of disodium phosphate and 14.3 g of citric acid in 400 ml of distilled water. The pH of the solution was adjusted to 3.2 by slowly adding 0.1 M NaOH and the volume of the buffer solution made to 1 litre using distilled water.
Standard stock solutions of Al, Cd, Pb, Ni and Zn were prepared from analar grade granulated metals. Each metal was first dried at 105 °C, cooled in a desiccator prior to weighing. For Al, 1.0g of accurately weighed aluminium metal was dissolved in 25 ml of concentrated HCl and diluted to 1 L to make 1000 ppm of stock solution. For Cd, 1.0 g of cadmium metal was accurately weighed and dissolved in 20 ml of 5 M HCl and diluted to 1 L using distilled water to make 1000 ppm standard stock solution. For Pb, 1.0 g of pure lead was accurately weighed and dissolved in 50 ml of 2 M nitric acid then diluted to 1 L to make 1000 ppm standard stock solution. For Ni, 1.0 g of nickel metal was accurately weighed and dissolved in 20 ml of concentrated nitric acid then made to 1 L of solution to make 1000 ppm standard stock solution. Zinc stock solution was prepared by dissolving 1.0 g of zinc metal in 40 ml of 1:1 Hydrochloric acid and water and diluted to 1 L to make 1000 ppm.

3.6 Method validation procedures

3.6.1 Calibration curves

For NO₃⁻ ion, freshly prepared standard stock solution was serially diluted to make 0.2, 0.5, 1.0, 1.5, 2.5 and 3.0 ppm standard solutions. While for NO₂⁻, from the fresh standard stock solution 0.5, 1.0, 1.5, 2.0, 3.0 and 5.0 ppm standard solutions were prepared. For Al, the freshly prepared standard stock solution was serially diluted to 0.5, 1.0, 1.5, 2.0, 5.0, 10.0 and 15.0 ppm standards. The standard stock solution for Cd was diluted to 0.5, 1.0, 1.5, 1.8 and 2.0 ppm standard solutions. For Pb, the standard stock solution was serially diluted to 0.1, 0.5, 1.0, 1.5, 2.0, 3.0 and 5.0 ppm standards. The standard stock solution for Ni was serially diluted to 0.5, 1.0, 1.5, 2.0, 3.0 and 4.0 ppm standards while
for Zn, the following standard solutions were prepared from the standard stock solution by serial dilution 1.0, 5.0, 10.0, 20.0 and 50.0 ppm.

The standard solutions were run in the instruments to obtain calibration curves. Each standard was run in triplicate and the resulting absorbance plotted against the concentration of the standard. The standard solutions were labeled appropriately and stored in plastic bottles for further calibration of the instruments during sample measurements. The concentration range of the standards and the optimum working range of the instruments are shown in Table 3.1.

<table>
<thead>
<tr>
<th>Instrument Used</th>
<th>Element/Ion</th>
<th>Concentration range of standards (ppm)</th>
<th>Optimum working range (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISE</td>
<td>Nitrate</td>
<td>0.2-3.0</td>
<td>0.1-1.0</td>
</tr>
<tr>
<td>ISE</td>
<td>Nitrite</td>
<td>0.5-5.0</td>
<td>0.1-1.0</td>
</tr>
<tr>
<td>AAS</td>
<td>Aluminium</td>
<td>0.5-15.0</td>
<td>1.0-4.0</td>
</tr>
<tr>
<td>AAS</td>
<td>Cadmium</td>
<td>0.5-2.0</td>
<td>0.1-1.0</td>
</tr>
<tr>
<td>AAS</td>
<td>Lead</td>
<td>0.1-5.0</td>
<td>1.0-2.0</td>
</tr>
<tr>
<td>AAS</td>
<td>Nickel</td>
<td>0.5-4.0</td>
<td>0.1-2.0</td>
</tr>
<tr>
<td>AAS</td>
<td>Zinc</td>
<td>1.0-50.0</td>
<td>0.4-1.6</td>
</tr>
</tbody>
</table>

3.6.2 Recovery Tests

The accuracy of ISE and AAS was investigated by repeatedly spiking samples with known amount of standards and calculating the percentage recovery using the formula shown in Equation 3.1.
\[ R = \left( \frac{a-b}{c} \right) \times 100 \] \hspace{1cm} \text{Equation 3.1}

Where

- \( R \) - Recovery
- \( a \) - Concentration of the sample after spiking
- \( b \) - Concentration of sample before spiking
- \( c \) - Concentration of standard used for spiking.

For the ISE, samples of known \( \text{NO}_3^- \) and \( \text{NO}_2^- \) concentrations were spiked with 15 ppm and 10 ppm standards respectively. For the AAS, samples of known Al, Cd, Pb, Ni and Zn concentrations were spiked with 5 ppm, 4 ppm, 10 ppm, 20 ppm, and 10 ppm standard solutions respectively.

3.7 Sample digestion and analysis

3.7.1 Nitrates and nitrites

From each tin 1.0 g of infant formula milk was accurately weighed and dissolved in de-ionized water and the volume made to 100 ml, 25 ml of the solution was accurately measured and placed into a 100 ml plastic beaker where 25 ml of the buffer solution was added. The solution was stirred with a magnetic stirrer then ISE electrode immersed and its reading allowed to stabilize before being recorded. ISE meter model: 290A connected to \( \text{NO}_3^- \) ISE and then \( \text{NO}_2^- \) ISE was used for analysis of concentration of nitrates and nitrites respectively. The measurements were done in triplicates. Calibration of the ISE meter using the standards was frequently done in between sample measurements. The operating conditions for ISE were as shown in the Table 3.2.
Table 3.2: The ISE operating conditions

<table>
<thead>
<tr>
<th>Specification</th>
<th>NO₃ ISE</th>
<th>NO₂ ISE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Voltage range indication (V)</td>
<td>1.36-1.66</td>
<td>0.25-0.75</td>
</tr>
<tr>
<td>Concentration range (ppm)</td>
<td>0.1-1400</td>
<td>0.5-500</td>
</tr>
<tr>
<td>Resolution with 12 bits ADC %</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td>pH range</td>
<td>2.5-11</td>
<td>2.5-11</td>
</tr>
</tbody>
</table>

(Khandapur, 2007)

3.7.2 Metals

From each tin 2.5 g of infant formula milk was accurately weighed into a Kjeldahl flask, 15 ml of concentrated nitric acid and 5 ml of 10% hydrogen peroxide were added and the resulting solution heated until no more brown fumes were produced. The resulting matrix was filtered through Whatman paper no 1 into 50 ml volumetric flask and its volume topped up with deionized water to the mark. The measurements for Al, Cd, Pb, Ni and Zn were done in triplicates using computerized Varian Atomic absorption Specrometer (Model: AA-10). Blank samples were digested following the same procedures as the samples and run in the instrument.

Their absorbance were recorded, the mean absorbance and standard deviations were determined and used for calculating limit of detection using Equation 3.2.

\[
\text{Limit of detection} = 3 \times \text{standard deviation of blank readings} \quad \text{Equation 3.2}
\]

\[
\text{Absorbance of standard} - \text{mean absorbance of blanks}
\]

Standards were run before and after a sample solution for precision. The operating conditions for AAS were as shown in the Table 3.3

Table 3.3: The AAS operating conditions
<table>
<thead>
<tr>
<th>Operating parameters</th>
<th>Al</th>
<th>Cd</th>
<th>Pb</th>
<th>Ni</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength (nm)</td>
<td>309.3</td>
<td>228.8</td>
<td>217</td>
<td>232.0</td>
<td>319.9</td>
</tr>
<tr>
<td>Slit width (nm)</td>
<td>0.5</td>
<td>1.0</td>
<td>0.2</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Flame type</td>
<td>N\textsubscript{2}O-acetylene</td>
<td>Air–acetylene</td>
<td>N\textsubscript{2}O-acetylene</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxidant flow rate (l/min)</td>
<td>4.5</td>
<td>1.5</td>
<td>4.5</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>Sensitivity (ppm)</td>
<td>8.4</td>
<td>0.011</td>
<td>0.055</td>
<td>0.066</td>
<td>0.33</td>
</tr>
<tr>
<td>Detection limit (ppm)</td>
<td>0.02</td>
<td>0.005</td>
<td>0.008</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Lamp current (mA)</td>
<td>6</td>
<td>5</td>
<td>8</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Optimum working range (ppm)</td>
<td>1.0-50.0</td>
<td>0.5-2.0</td>
<td>2.0-8.0</td>
<td>3-12</td>
<td>1.0-50.0</td>
</tr>
</tbody>
</table>

(Khandapur, 2007)

3.8 Data analysis and presentation

Mean values obtained for NO\textsubscript{3}\textsuperscript{-}, NO\textsubscript{2}\textsuperscript{-}, Al, Cd, Pb, Ni and Zn in the seven brands of infant formula milk samples were compared by One-Way ANOVA at 95% level using SPSS 18 for windows assuming that there were significant differences among them when the statistical comparison gave $p < 0.05$. The EWI per body weight of the infant for the ions and metals in each brand of formula milk were then calculated based on the feeding table provided in each brand of formula milk (0-6 months) using Equation 3.3.

EWI = \frac{\text{concentration of element in } \mu g/mg \times \text{av mass of formula intake per wk in mg}}{\text{Average mass of infant in kg}} . \text{Equation 3.3}

The results are presented in tables and figures in chapter four.

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Introduction

The levels of NO\textsubscript{3}\textsuperscript{-} and NO\textsubscript{2} in the seven purposively sampled brands of formula milk for infants aged 0-6 months sold in a major store in Nairobi were determined by ISE meter.
The levels of Al, Cd, Pb, Ni and Zn were determined by AAS. Results obtained are presented in tables and figures and further discussed.

4.2 Method validation

4.2.1 Calibration curves and correlation coefficients

For ISE calibration curves were obtained by plotting current against concentration of the standard solutions while for AAS absorbance was plotted against corresponding concentrations with optimized instrument conditions. Calibration curve for aluminium is as shown in figure 4.1 while calibration curves for nitrate, nitrite ions and other metals are in the appendices.

Figure 4.1: Calibration curve for aluminium standards

Regression analysis was used to evaluate the linearity of ISE and AAS results using the established calibration curves. The correlation coefficients and the equations for the calibration curves are given on Table 4.1

Table 4.1: Correlation coefficients and equations of calibration curves for ISE and AAS

<table>
<thead>
<tr>
<th>Instrument Used</th>
<th>Ion/Element</th>
<th>Correlation coefficient ($r^2$)</th>
<th>Equation of calibration Curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISE</td>
<td>Nitrate</td>
<td>0.9999</td>
<td>$y=4.7063x+0.0309$</td>
</tr>
<tr>
<td></td>
<td>Nitrite</td>
<td>0.9979</td>
<td>$y=0.0579x+0.0241$</td>
</tr>
<tr>
<td>AAS</td>
<td>Aluminium</td>
<td>0.9998</td>
<td>$y=0.004x+0.0002$</td>
</tr>
<tr>
<td></td>
<td>Cadmium</td>
<td>0.9998</td>
<td>$y=0.0318x+0.0018$</td>
</tr>
<tr>
<td></td>
<td>Lead</td>
<td>0.9993</td>
<td>$y=0.0238x-0.004$</td>
</tr>
<tr>
<td></td>
<td>Nickel</td>
<td>0.9988</td>
<td>$y=0.0607x+0.0037$</td>
</tr>
<tr>
<td></td>
<td>Zinc</td>
<td>0.9998</td>
<td>$y=0.064x+0.0076$</td>
</tr>
</tbody>
</table>
The $r^2$ values indicate that the calibration curves are linear over the concentration range of the standards thus ISE and AAS techniques are expected to have given precise and accurate results (Duan et al., 2003).

### 4.2.2 Recovery test

The concentration of unspiked samples, the recovered concentration of ions and metals and the percentage recovery are shown in Table 4.2

**Table 4.2: Percentage recovery for ISE and AAS**

<table>
<thead>
<tr>
<th>Ion/Metal</th>
<th>Concentration in ppm</th>
<th>Unspiked sample</th>
<th>Spiked amount</th>
<th>Recovered</th>
<th>Percentage recovery % R±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate</td>
<td>0.02±0.05</td>
<td>15.00</td>
<td>14.90±0.12</td>
<td>99.20±0.80</td>
<td></td>
</tr>
<tr>
<td>Nitrite</td>
<td>0.0±0.0</td>
<td>10.00</td>
<td>9.94±0.02</td>
<td>99.40±0.70</td>
<td></td>
</tr>
<tr>
<td>Aluminium</td>
<td>0.52±0.04</td>
<td>5.00</td>
<td>5.48±0.02</td>
<td>99.20±0.15</td>
<td></td>
</tr>
<tr>
<td>Cadmium</td>
<td>0.00±0.00</td>
<td>4.00</td>
<td>3.98±0.01</td>
<td>99.50±0.27</td>
<td></td>
</tr>
<tr>
<td>Lead</td>
<td>0.02±0.01</td>
<td>10.00</td>
<td>10.1±0.06</td>
<td>99.90±0.33</td>
<td></td>
</tr>
<tr>
<td>Nickel</td>
<td>0.03±0.01</td>
<td>20.00</td>
<td>20.01±0.07</td>
<td>99.90±0.40</td>
<td></td>
</tr>
<tr>
<td>Zinc</td>
<td>9.30±0.13</td>
<td>10.00</td>
<td>19.11±0.43</td>
<td>98.10±0.50</td>
<td></td>
</tr>
</tbody>
</table>
From Table 4.2, the percentage recovery for the ions and metals was between 98.1% - 99.9%, this confirms that both the ISE and AAS procedures used are of good precision (Duan et al., 2003).

### 4.3 Levels of nitrates and nitrites in infant formula milk

The concentration of nitrate and nitrite seven brands of formula milk for infants aged 0-6 months sold in Nairobi County was determined using ISE. The results obtained are presented in Table 4.3.

<table>
<thead>
<tr>
<th>Brand code</th>
<th>Concentration in µg/mg (±SE); n=9</th>
<th>NO$_3^-$</th>
<th>NO$_2^-$</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>0.031±0.003$^{bc}$</td>
<td></td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>(0.024-0.042)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>U</td>
<td>0.022±0.004$^a$</td>
<td></td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>(0.020-0.023)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>0.034±0.008$^{bc}$</td>
<td></td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>(0.030-0.037)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>W</td>
<td>0.033±0.001$^{bc}$</td>
<td></td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>(0.029-0.040)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>0.031±0.002$^{bc}$</td>
<td></td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>(0.025-0.040)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Y</td>
<td>0.027±0.005$^{ab}$</td>
<td></td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>(0.025-0.039)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z</td>
<td>0.039±0.004$^c$</td>
<td></td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>(0.026-0.055)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean values followed by the same small letter in the same column are not significantly different.
Ranges in parenthesis  
ND- Not detected  
(One way ANOVA, α =0.05 SNK test)

The results presented in Table 4.3 are discussed in the following sub sections.

4.3.1 Levels of nitrates in infant formula milk

From Table 4.3, all the seven brands of infant formula milk were found to contain some levels of nitrate ion. This ranged from $0.022\pm0.004$ (µg/mg) in brand U to $0.039\pm0.004$ (µg/mg) in brand Z. These levels were found to differ significantly between the different brands ($p <0.001$). Although not within scope of this study, this difference can be attributed to the different sources of raw materials processed to manufacture the different brands (Tai et al., 2013). The levels of nitrates in the different brands of infant formula milk reported in this study were below the WHO limits and do not pose safety risks to the infants. The levels were also below those reported in other studies. Reinik (2005) reported level of nitrate in infant formula milk in Estonia as 2 µg/mg while Tai et al. (2013) observed that the level of nitrate in different brands of infant formula milk in Taiwan was 0.3 - 83.2 µg/mg. They observed that these levels were below the WHO limits and did not pose food safety risks. However, they recommended that investigation of nitrate content in infant formula milk should be done as a safety precaution.

Nitrates have found use as food preservatives and this explains their presence in infant formula milk (WHO, 2006). The main toxicological concern for nitrate exposure to infants is associated with its conversion to nitrite before or after reaching the body. The nitrite ion oxidizes Fe$^{2+}$ in haemoglobin to Fe$^{3+}$ rendering it unable to carry oxygen.
resulting into lack of oxygen in the body tissue, a condition known as methemoglobinemia which can cause adverse health problems to the infant including death (Tamme, 2006).

4.3.2 Levels of nitrites in infant formula milk

From Table 4.3, nitrite ion was not detected in all the seven brands of infant formula milk. However, infant formula milk is a possible source of exposure of nitrite ion to infants since the water used to prepare the infant formula milk may be contaminated with nitrite ion. Tai et al. (2013) reported that the levels of nitrite in different brands of infant formula milk in Taiwan were below the limit of detection. Reinik (2005) reported that the level of nitrite in infant formula milk in Estonia was about 0.03 µg/mg. He noted that these levels were below the set limit of nitrite in infant formula milk and thus did not pose a health risk to the infants.

While these results do not warrant fear of dangers of nitrite in infant formula milk, the water used to prepare the milk can be a possible source of nitrite contamination. The nitrite ion oxidizes Fe$^{2+}$ in haemoglobin to Fe$^{3+}$ rendering it unable to carry oxygen resulting into lack of oxygen in the body tissue. Nitrite ion may also react with secondary amine to form N-nitrosoamine which is suggested as a possible carcinogen (Tamme, 2006).
4.4 Levels of Al, Cd, Pb, Ni and Zn in infant formula milk

The levels of Al, Cd, Pb, Ni and Zn in seven brands of formula milk for infants aged 0-6 months sold in Nairobi County was determined using AAS. The results obtained are presented in Table 4.4
Table 4.4: Mean concentration of selected metals in infant formula milk

<table>
<thead>
<tr>
<th>Brand code</th>
<th>Concentration in µg/mg (X ±SE); n=9</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Al</td>
</tr>
<tr>
<td>T</td>
<td>1.054±0.085&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(0.828-1.394)</td>
</tr>
<tr>
<td>U</td>
<td>2.069±0.447&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(0.940-3.840)</td>
</tr>
<tr>
<td>V</td>
<td>1.545±0.089&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(1.298-1.946)</td>
</tr>
<tr>
<td>W</td>
<td>1.099±0.068&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(0.924-1.380)</td>
</tr>
<tr>
<td>X</td>
<td>2.156±0.423&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(1.132-3.842)</td>
</tr>
<tr>
<td>Y</td>
<td>1.543±0.245&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(0.770-2.560)</td>
</tr>
<tr>
<td>Z</td>
<td>1.405±0.031&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(1.302-1.602)</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Mean values followed by the same small letter in the same column are not significantly different.
Ranges in parenthesis
ND- Not detected
(One way ANOVA, α =0.05 SNK test)
The results presented in Table 4.4 are discussed in the following sub sections.

4.4.1 Levels of Al in infant formula milk

From Table 4.4 all the seven brands of infant formula milk were found to contain some levels of Al. Brand code T had the lowest level; 1.054±0.085 µg/mg while brand code X had the highest level; 2.156±0.423 µg/mg. The levels in most of the brand code were found to differ significantly (p<0.001). The difference can be attributed to the different manufacturing practices, quality of raw materials and packaging containers used (Khalifa and Ahmad, 2010). Though all the brands were found to contain some amount of Al, the labels do not show these levels. This is a concern as aluminium toxicity in infants is well documented. Aluminium toxicity can lead to reduced skeletal mineralization observed in preterm infants and infants with growth retardation. At high concentrations Al can cause neurotoxicity (Pennington, 1995; Banks and Kastin, 2008). Increased Al deposits in the brain can lead to risk of Alzheimer’s disease (Hawkes, 2006; Ferreira et al., 2008; Rondeau et al., 2008).

The levels of Al in most brand codes reported in this study were similar to levels of Al from other studies. Khalifa and Ahmad (2010) reported the mean concentration of infant formula milk (0-6 months) sold in Saudi Arabia as 1.944±1.09 µg/mg while Salah (2012) reported levels of Al in infant formula milk sold in Egypt as 1.350±0.036 µg/mg. Tasneem et al. (2009) reported the levels of Al in infant formula milk in Pakistan ranged between 1.070 – 2.170 µg/mg. They observed that the levels do not raise fears of Al toxicity as they are below the set WHO limits. However they recommended investigation
of Al levels in infant formula milk since infant formula milk provides an exposure route for Al to infants.

### 4.4.2 Levels of Cd in infant formula milk

From Table 4.4 cadmium was not detected in all the brands of infant formula milk. However, infant formula milk is a possible source of exposure of cadmium to infants since the water used to prepare the infant formula milk may be contaminated with cadmium (Khalifa and Ahmad, 2010). Other studies have reported concentration of cadmium in infant formula milk. Tasneem et al. (2009) reported the levels of Cd in infant formula milk in Pakistan to range between 0.010 – 0.0344 µg/mg. Salah (2012), studied the levels of toxic metals in infant formula milk in Egypt and reported cadmium concentrations of 0.0210 ± 0.016 µg/mg. Behrooz et al. (2009) studied levels of selected metals in infant formula milk in Iran and reported concentrations of cadmium in infant formula milk as 0.0359 ± 0.215 µg/mg. Anna (2009) conducted an assessment of infant exposure to lead and cadmium through infant formula milk in Lublin and reported cadmium concentration range of 0.0004-0.02 µg/mg in different brands of infant formula milk. However, the intake levels of Cd from these concentrations were below the WHO limits.

Cadmium is toxic even at trace concentrations (Bearer, 2000). Exposure to cadmium has been associated with anaemia, osteomalacia, cardiovascular diseases and kidney failure (Berglund et al., 2000). Cadmium toxicity targets the kidney and bone which are crucial
calcium metabolizing sites thus leading to a decrease in the amount of calcium in blood (Gaw et al., 2006).

### 4.4.3 Levels of Pb in infant formula milk

From Table 4.4 all the seven brands of infant formula milk were found to contain some levels of Pb, these ranged from 0.018±0.001 µg/mg in brand code V to 0.059±0.002 µg/mg in brand code W. The levels brand codes T, U, V and Y were found not to differ significantly (p<0.001). The level in brand code W differed significantly from the levels in other brand codes. The difference can be attributed to the different manufacturing practices and quality of raw materials used (Khalifa and Ahmad, 2010)

Except brand codes T and V, all the other brand codes had levels of Pb above the KEBS maximum limit of 0.02 ppm. This raises a serious health concern for infants fed on these brand codes. Despite all the brand codes investigated having some amounts of Pb, the labels on these brands do not show these levels, the public need to be aware of the presence of Pb in the infant formula milk sold in Nairobi County.

The levels of Pb reported in this study are comparable to the levels reported in other studies. Behzoor et al. (2009) reported that the levels of Pb in infant formula milk in Iran was 0.384±0.222 µg/mg. Tasneem et al. (2009) reported the levels of Cd in infant formula milk in Pakistan to range between 0.010 – 0.0344 µg/mg, Khalifa and Ahmad (2010) reported that the average concentration of Pb in infant formula milk sold in Riyadh was 0.018±0.002 while Anna (2009) reported concentration range of levels of Pb in different brands of infant formula milk in Lublin as 0.094-0.450 µg/mg. They
observed that though the levels were below the set limits, presence of Pb in infant milk was of great concern and all measures should be taken to reduce its levels since it can accumulate to dangerous levels since children depend on milk at this stage.

Lead is rapidly absorbed into the blood stream and has adverse effects on the central nervous system, cardiovascular system and the immune system (Bergesson and Lynn, 2008). Exposure of infants to lead even at trace quantity can cause retardation in IQ, interference with growth and development of the infant, attention deficit disorder, speech delay, hyperactivity and lower class standing (Nevin, 2000; Karin and Terry, 2004; Lanphear et al., 2008).

4.4.4 Levels of Ni in infant formula milk

From Table 4.4, all the seven brands of infant formula milk investigated in this study were found to contain some levels of Ni, these ranged from 0.022±0.001 µg/mg in brand code X to 0.032±0.002 µg/mg in brand code W. Except for brand code X, the levels in all the other brand codes did not differ significantly (p<0.001). The labels however do not show these levels.

The levels on Ni reported in this study are comparable to levels reported in other studies. A study by Food Standards Agency in United Kingdom in 2006 reported the mean concentration of nickel in various brands of infant formula milk as 0.1 µg/mg. Michael et al. (2000) reported that the concentration of nickel in some infant formula milk sold in
Austria was 0.0179 \mu g/mg. Though these levels are below the set limit, presence of Ni in water used to prepare infant formula milk can cause Ni toxicity in infants.

In small quantities, nickel is essential and serves as a cofactor or structural component of specific metalloenzymes with a variety of physiologic functions. Nickel has been shown to facilitate ferric iron absorption or metabolism (Robert, 2004). However, nickel has several adverse health effects when taken in large quantities such as decrease in infant weight, dizziness, allergic reactions and can cause to reduced lung function (Reilly, 2002). Nickel and certain nickel compounds have been listed by the National Toxicology Program (NTP) as being reasonably anticipated to be carcinogens (Ballatori, 2002).

### 4.4.5 Levels of Zn in infant formula milk

From Table 4.4 all the seven brands of infant formula milk investigated in this study were found to contain some amount of Zn, these ranged from \(24.00\pm0.52\ \mu g/mg\) in brand code T to \(32.04\pm0.74\ \mu g/mg\) in brand code U. The levels of Zn in the different brand codes differed significantly \((p<0.001)\). The difference can be attributed to the different manufacturing practices and quality of raw materials used (Khalifa and Ahmad, 2010). Zn is among the elements listed in the packaging labels of the infant formula milk. The levels listed in the labels for brand codes T, U, V, W, X, Y, and Z are 40, 55, 36, 36, 48, 34 and 36 \mu m/mg respectively. The levels listed in the labels of the tins of the infant formula milk however were below those reported in this study which may result to Zn deficiency for the infants. These levels are below the maximum limit set by KEBS of 43 \mu g/mg and therefore do not pose fear of toxicity.
Other studies on levels of Zn in infant formula milk other countries have reported varying concentrations of Zn. Milena et al. (2009) studied the levels of essential elements in infant formula milk in Brazil reported concentration of zinc as $48.6 \pm 0.1 \mu g/mg$. Abua et al. (2002) studied the concentration of various elements in infant formula milk in United States, Nigeria and United Kingdom and reported the mean concentration of zinc as 49.4 µg/mg. Khalifa and Ahmad (2010) in a study of levels of key elements in commercially available infant formula in Saudi Arabia reported mean concentrations of zinc in the infant formula milk as $35.7 \pm 0.833 \mu g/mg$. Ljung et al. (2011) studied concentration of various elements in infant formula milk in Sweden and reported concentration of Zn as $51.0 \pm 0.04 \mu g/mg$. This difference can be as a result of some infant formula milk being fortified with Zn (Milena et al., 2009).

Zinc is a vital ingredient in infant formula milk. Intake of higher or lower amounts of Zn can affect the health of an infant (Milena et al., 2009). It plays an important role as an integral part of coenzymes related to synthesis and metabolism of proteins and nucleic acids (Picciano, 2001). Zinc deficiency can occur due to insufficient intake or low bioavailability in the diet, this can lead to an impairment of DNA, growth retardation and harms immune function. However, high concentration of zinc in infant formula milk may cause vomiting, renal damage (Doherty et al., 2011).

4.5 Estimated weekly intake of nitrate and metals from of infant formula milk

The estimated weekly intake (EWI) of nitrate and metals investigated in this study for infants feeding on the different brands of infant formula milk was calculated on the basis
of feeding tables provide by the manufactures and the levels of nitrate and elements reported in this study. The results were as shown in the Table 4.5. In line with the results, nitrite and cadmium were not detected in the brands of infant formula milk investigated in this study hence their EWI were not calculated.

Table 4.5: Estimated weekly intake of nitrates and metals by infants

<table>
<thead>
<tr>
<th>Brand Code</th>
<th>Estimated Weekly intake (µg/kg of infant body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO\textsubscript{3}</td>
</tr>
<tr>
<td>T</td>
<td>1670</td>
</tr>
<tr>
<td>U</td>
<td>1530</td>
</tr>
<tr>
<td>V</td>
<td>2360</td>
</tr>
<tr>
<td>W</td>
<td>1170</td>
</tr>
<tr>
<td>X</td>
<td>4600</td>
</tr>
<tr>
<td>Y</td>
<td>3400</td>
</tr>
<tr>
<td>Z</td>
<td>2800</td>
</tr>
<tr>
<td>PTWI</td>
<td>23800</td>
</tr>
</tbody>
</table>

From Table 4.5, the EWI of NO\textsubscript{3}, Al, Pb, and Zn were below the PTWI set by the Joint Committee on Food Additives of the Food and Agriculture Organization and the EU Scientific Committee for infant feeding showing safety of the infant formula milk. These values were lower than those reported from other studies. Ljung et al. (2011) in a study of concentration of essential and toxic elements in infant foods in Sweden reported the EWI of Cd, Pb and Zn for infants aged 6 weeks fed on infant formula milk as 0.77 µg/kg of body weight (bw), 5.95 µg/kg of bw and 30,100 µg/kg of bw respectively. They observed that the intake of essential and toxic elements was higher for infant fed on infant formula milk than breast fed infants. Therefore infants fed on infant formula milk were at a risk of toxicity by such elements as lead and cadmium. Khalifa and Ahmad (2010) reported EWI
for Al, Cd and Pb for infants feeding on formula milk in Saudi Arabia as 217 µg/kg of bw, 0.78 µg/kg of bw and 2.10 µg/kg of bw respectively. However, many infants feeding on infant formula milk receive more portions of the infant formula milk than is stipulated in the feeding guidelines given by the manufacturer hence the EWI may be higher than the values reported (Gian et al., 2009).

CHAPTER FIVE: CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Levels of nitrate, nitrite, aluminium, cadmium, lead, nickel and zinc in purposively seven brands of formula milk for infants aged 0-6 months sold in Nairobi County were determined using ISE and AAS techniques. The following conclusions can be made.
(i) All the brands of infant formula milk had levels of nitrate although these were below the recommended limits.

(ii) The findings indicated none detectable levels of nitrite and cadmium.

(iii) Aluminium, lead and nickel were detected in the seven brands of infant formula milk but they were below the limits set by WHO. However, there is still a concern considering the likelihood of metal bioaccumulation which can lead to adverse health effects in infants.

(iv) The levels of Pb in five of the brands were higher than the maximum limit of KEBS, this is a cause of concern due to the adverse health effects associated with lead.

(v) The levels of Zn in all the brands of infant formula milk were below the levels indicated in the labels. This is of concern since it can result into Zn deficiency.

5.2 Recommendations from this study

(i) Exclusive breastfeeding for the first six months of infancy should be emphasized. In event that it is not possible to exclusively breastfeed, infant formula milk may be used with caution noting the risk of bioaccumulation.

(ii) There should be stricter enforcement of KEBS regulations to ensure that manufacturers of infant formula milk comply.

(iii) Manufacturers of infant formula milk should be compelled to provide detailed labels of essential and non essential ions and metals present in the infant formula milk.
5.3 Recommendations for further work

(i) Levels of nitrate, nitrite and the toxic metals in infant formula milk marketed for use by infants beyond 6 months should be studied.

(ii) Levels of essential, non essential and toxic substances in other baby foods used as substitutes or complements of breast milk should be studied.

(iii) Water that is used to reconstitute the infant formula milk should be analyzed for levels of nitrate, nitrites and toxic metals.

REFERENCES


APPENDICES

Appendix 1: Calibration curve for nitrate
Appendix 2: Calibration curve for nitrite
Appendix 3: Calibration curve for cadmium

Appendix 4: Calibration curve for lead
Appendix 5: Calibration curve for nickel
Appendix 6: Calibration curve for zinc
Appendix 7: Letter of grant award

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When replying please quote
secretary@ncst.go.ke

P.O. Box 30623-00100
Nairobi-Kenya
Website: www.ncst.go.ke

Our Ref: NCST/ST&I/RCD/4th Call M.Sc/195

Date: 2nd October, 2012

Vincent Otieno Odhiambo
Kenyatta University
P.O. Box 43844
NAIROBI

RE: SCIENCE, TECHNOLOGY AND INNOVATION RESEARCH GRANT (M.Sc)

I'm pleased to inform you that, you have been awarded the Science, Technology and Innovation (ST&I) grant for your M.Sc research proposal.

National Council for Science and Technology (NCST) has approved an amount of Kenya shillings one hundred and sixty thousand (Ksh. 160,000) towards your proposal entitled “Determination of selected metals, nitrates and nitrites in some infant formula milk in Nairobi County, Kenya”. The approved grant is for research costs only and must not be used for payment of tuition fees.

Find the enclosed Research Grant Contract Form (NCST/ST&I/CONTRACT/FORM 1C) that should be duly completed and returned to NCST. You should attach a copy of your national identity card and acceptance letter. Your contract must be accompanied by an abstract of your project, not more than 500 words and a recent digital passport size photograph. A soft copy of your abstract and passport size photograph must be submitted in (MS Word format) to the email:- postgraduate@ncst.go.ke

Your signed contract form, acceptance letter and abstract should reach us not later than 12th October, 2012 for further action.

PROF. SHAUKAT ABDULRAZAK, Ph.D, FSB, FASI, MBS.
SECRETARY/CEO

cc: Vice Chancellor
Kenyatta University