SELENIUM, MANGANESE, ZINC, AND COPPER CONTENT IN INFANT FORMULA

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CHAPTER I

INTRODUCTION

Background

Infancy is probably the most nutritionally demanding period of life. Weight typically doubles during the first 4 to 6 months and triples by the end of the first year (1). At this rate of growth, adequate nutrient intake is a must. Infants generally get their diet solely from one food source which may be human milk, commercial infant formulas, or both.

The importance of trace elements has been recognized for a few decades. They have very important functions in growth and development. During infancy especially, it is very important to ensure that appropriate levels of utilizable essential trace elements are provided. Elements that fall under this trace element specification are : magnesium, iron, zinc, copper, iodine, selenium, chromium, manganese, molybdenum and fluorine. Most of the requirement for each element is measured as micrograms (μg) instead of milligrams (mg) or grams (g).

The Problem

Breast milk is considered to be the ideal food for infants. It provides a complete nutrient profile as well as immune system components for the first few months of the infant's life (1). But the popularity of bottle feeding newborn infants has increased dramatically in the last 50 years, mostly in developing countries, but in the United States as well.

Few data are available concerning the amount or the bioavailability, of some trace elements contained in infant formulas. Does infant formula provide insufficient or excessive amounst of these elements? We do know that infant formula does not provide the immunity protection that human colostrum does.

Infant formulas are relatively expensive. Its use in developing countries can be a some disadvantage to infants, not only physically tbut also economically to the parents of the infants. Some normal healthy mothers actually prefer to bottle feed their infants with infant formula instead of their natural milk because they think that infant formula is much better in quality compared to breast milk. It is very important to give the society complete information about the advantages and disadvantages of infant formula, and providing complete data concerning the trace elements is one step (3).

Purpose of the Study

The purpose of this research was to estimate the content of copper, zinc, selenium and manganese, in several kinds of infant formulas manufactured by Ross Laboratories (Columbus, Ohio), to contribute to a data bank to establish ranges of these trace elements and to determine whether the infant formulas provide them within the ranges normally found in human milk.

Objectives of the Study

This study was designed to investigate the following objectives :

1. Determine selenium, manganese, zinc, and copper concentration in selected infant formulas.
2. Accumulate data in order to help establish ranges of selenium and manganese in infant formula.
3. Compare the value of copper and zinc content in infant formulas in the study with the value reported by the manufacturer.

4. Compare the data with the copper, zinc, selenium and manganese concentration of milk from a normal healthy mother in her child bearing age.
5. Compare the data with the copper, zinc, selenium, and manganese recommendations for infants.

The design of this study was based on the following assumptions :

1. The concentrations of selenium, manganese, zinc and copper in infant formula will be higher compared to that in breast milk of a normal, healthy mother of child bearing age.
2. The infant formula content of selenium and manganese, zinc and copper are within the range established in the Estimated Safe and Adequate Daily Dietary Intake (ESADDI) or Recommended Dietary Allowance (RDA).

Scope and Limitation

Some limitations that may be present in this research :

1. Human error of inaccuracy during
   a. weighing the samples.
   b. measuring the volume of the samples.
   c. adding nitric acid and hydrogen peroxide to the samples.
2. Possible contamination during
   a. cleaning and preparing the tubes for the sample.
   b. adding nitric acid and peroxide to the sample.
   c. diluting the sample.
   d. analyzing the sample.

CHAPTER II

REVIEW OF LITERATURE

Modified and commercially manufactured infant formulas prepared from cow's milk, soy bean, and various cereals are widely available and used for infant feeding. Four major companies that supply this large demand for infant formula are Wyeth, Mead Johnson, Carnation, and Ross Laboratories.

Due to some concern about the composition of infant formulas, the Committee on Nutrition of the American Academy of Pediatrics issued a policy statement to standardize these products (2). The standard of comparison uses breast milk composition from healthy mothers as its base (Table I).

Some of the18 trace minerals have been shown to have major roles in normal human body function, and they are magnesium, iron, zinc, copper, iodine, selenium, chromium, manganese, molybdenum and fluorine. Data are available for only magnesium, iron, zinc, copper, and iodine in the commercially available cow's milk and soy milk based formula manufactured by Ross Laboratories (1).

This study examines four trace minerals (selenium, manganese, zinc and copper) in several kinds of infant formulas manufactured by Ross Laboratories. The formulas are SIMILAC® with Iron infant formula (ready to feed, concentrated, and powder form), SIMILAC® PM 60/40 Low Iron infant formula (ready to feed and powder form), ALIMENTUM® Protein Hydrolysate infant formula with iron (ready to feed form), SIMILAC® Low Iron infant formula (ready to feed, concentrated, and powder form), ISOMIL® Soy Formula with Iron (ready to feed, concentrated, and powder form), and SIMILAC® Special Care infant formula with iron
(ready to use form). The result will be compared to the composition of milk from a healthy mother.

Table I. Estimates of Concentration of Nutrients in Mature Human Milk

Nutrient	Amount in Human Milk (a)
	g/liter ± SD (b)
Lactose	72.0 ± 2.5
Protein	10.5 ± 2.0
Fat	39.0 ± 4.0
	mg/liter ± SD
Calcium	280 ± 26
Phosphorus	140 ± 22
Magnesium	35 ± 2
Sodium	180 ± 40
Potassium	525 ± 35
Chloride	420 ± 60
Iron	0.3 ± 0.1
Zinc	1.2 ± 0.2
Copper	0.25 ± 0.03
Vitamin E	2.3 ± 1.0
Vitamin C	40 ± 10
Thiamin	0.210 ± 0.035
Riboflavin	0.350 ± 0.025
Niacin	1.5 ± 0.2
Vitamin B6	93 ± 8 (c)
Panthotenic acid	1.8 ± 0.2
	µg/liter ± SD
Vitamin A, RE	670 ± 200
Vitamin D	0.55 ± 0.10
Vitamin K	2.1 ± 0.1
Folate	85 ± 37 (d)
Vitamin B12	0.97 (e,f)
Biotin	4 ± 1
Iodine	110 ± 40
Selenium	20 ± 5
Manganese	6 ± 2

Table I. Estimates of Concentration of Nutrients in Mature Human Milk (continue).

Nutrient	Amount in Human Milk (a)
	µg/liter ± SD
Fluoride	16 ± 5
Chromium	50 ± 5
Molybdenum	NR (g)

a From Committee on Nutrition of the AAP 1985 (2).
b Standard Deviation.
c From Styslinger L, Kirksey A: American Journal of Clinical Nutrition 41:21,1985.
d From Brown CM: Journal of Pediatric of Gastroenterol Nutrition 5:278,1986.
e From Sandberg DP, James AB, Charles AH: American Journal of Clinical Nutrition 34:1717,1981.
f Standard Deviation is not reported; range 0.33 to 3.20.
g Not Reported.

The History of Infant Formula

According to Hunter (3), the major drive for infant formulas started in the mid 1800's, when there was a decline of wet nursing. The first company that manufactured evaporated milk was Borden Company in 1856. When the sterilization process was discovered in 1883, infant formulas were developed. This sterilization process modifies the casein curds and make it digestible by the infant's digestive track.

By 1930, infant formulas had become a giant and profitable enterprise that was aggressively promoted (3). During World War II when the number of women employed away from home increased, bottle feeding became a norm in United States and Western Europe.

Evaporated milk was the most widely used ingredient for infant formulas until 1950. By 1960, 80 % of all bottle-fed babies used evaporated milk. But after 1960, most evaporated milk was not used anymore and instead, it was replaced by commercial infant formulas (3). Today, there are hundreds of infant formula brands world wide. In United States, these companies dominate the market: Wyeth, Mead Johnson, Carnation, Ross Laboratory are among them.

Most infant formulas are based on cow's milk. Some of them use a protein base of soybean isolate for infants allergic to protein in cow's milk or for infants not able to digest it. For infants with an inborn error of metabolism, there are special formulas on the market.

Several problems arise from improper infant formula preparation, especially those which are not ready to use and need to be prepared. Feeding the infant with improperly prepared formula containing high sodium concentration creates an abnormal thirst. The infant will cry for more formula which, again, makes the infant more thirsty.
This excess sodium problem leads to overweight infants and has caused manufacturers to changed the composition of infant formula. A reduced amount of sodium became mandatory in 1974.

Differences Between Cow's Milk and Human Breast Milk and Some Problems Caused by Infant Formula

All mamals' milk are not only highly complex, but also very species specific. Cow's milk specifically matches needs for calves but not humans. The total protein of human milk consists of approximately 40% of whey and 60% of casein, whereas cow's milk consists of 20% whey and 80% casein (3). For human infants, whey is a more digestible protein as compared to casein. A lesser amount of casein in human breast milk will produce a much softer and flocculent curd that can be easily digested by the infant's digestive track. It is true that technology enables the manufacture to break down the size of the curd produced by cow's milk to make it more digestible, but the quality of the protein in human breast milk is better absorbed. Infants fed with infant formula will produce larger stools, and these stools carry some of the nutrients out of the body.

Infant formula also often has sugar (sucrose) in it, and the combination of this sugar and casein can cause necrotizing enterocolitis, a condition that can bring a fatal result in infants (3). As a matter of fact, up to 8,000 infants in United States are affected by this condition each year. In the premature infant, undigested sugar is fermented in the stomach and becomes acidic. Then it will trigger an inflammatory reaction that actually can break down the lining of the stomach. The final result can be intestinal rapture. Several symptoms of necrotizing enterocolitis are distended stomach, bloody diarrhea, fatigue, loss of appetite and lower body temperature.

Another problem faced by premature infants is that the soy in soy-based formula that is recommended for them might decrease iron uptake. Bone development / mineralization in these infants is less compare to other fed with cow's milk base formula (3). Not only that, soy-based formula is also deficient in iodine.

Some Problems Related to Trace Minerals in Infant

One case of aluminum toxicity in infants with abnormal kidney function has been

reported in the 1983 by Randell (4). Even though this might not be dangerous for a normal infant, there is no assurance whatsoever about its safety in a long term range.

In 1986, it was reported that the selenium content of infant formula was much lower compared to human breast milk (3). It is suggested that infant formulas should be supplemented by selenium. Due to the potential for selenium toxicity, supplementation should be done very cautiously.

In 1990, the Committee on Nutrition of the American Academy of Pediatrics recommended infant formulas to be fortified with iron even though in 1987 it was proven that iron fortification can depress copper absorption in infant (3). It has been recognized that copper content of human milk is two to three times higher than that of cow's milk.

The Increasing Trend Toward Bottle Feeding Instead Of Breast Feeding

As mentioned previously the increasing trend toward bottle feeding started in World War II when more women worked away from home (3). Since then, the trend has not subsided. More women are a single parent now to their children, and to be able to supporthem financially, they have to go to work. Short maternity leaves for these women discourage them to breast fed their infants. In many countries, including the United States, breast feeding infants in public is taboo. The number of designated places for breast feeding is actually increasing in the last 10 years, but it still is not enough to encourage working mothers to breast feed their infants.

Present Knowledge in Selenium, Manganese, Zinc, and Copper

Selenium

History

Selenium was discovered by Berzelius in 1817. It was recognize as a nutritionally essential element in 1951 by a physician from Germany named Klaus Schwartz. He

worked for National Institutes of Health, found an unrecognized factor which he called Factor 3, guards against necrotic liver degeneration. He later identified Factor 3 as selenium due to the strong garlic odor it produced. Its biological toxicity was discovered in 1957 (5).

Selenium's biopotency went unnoticed for many years due mainly to the trace amount needed to function and to the unavailability of such equipment at that time to detect that very small amount.

Roles and Function

A study by Dr. Yang in China brought some strong suggestion of the important function of selenium in Keshan disease (5), an endemic cardiomyopathy of unknown cause, during 1974-1977 in Sichuan province. A supplementation in a form of sodium selenite was given and proved to prevent further spread of the disease.

Selenoproteins have been identified: glutathione peroxidase, 5'-deiodinase type I (6), and selenoprotein P. In addition to that, selenium reportedly contains transport RNA (tRNA).

The first suggestion of an involvement of selenium and glutathione peroxidase (often abbreviated as GSH-Px) was stated by Tsen and Tappel in 1958 (7), where it was suggested that selenium was tightly bound to the enzyme and that glutathione peroxidase is a selenoprotein. The function of glutathione peroxidase in-vivo, primarily to metabolize hydrogen peroxide, thus protects against injury. The latest study by Vadhanavikit and Ganther in 1993 (6) mentions that glutathione peroxidase helps to protect the thyroid gland from hydrogen peroxide that is needed to synthesis tyroxine (T4), a thyroid hormone. When rats were fed with selenium-deficient diet, the activity of glutathione peroxidase in liver and plasma was reduced to less than 1% (8). This means that the measurement of glutathione peroxidase activity can be used in assessing selenium nutritional status in human.

Another selenoprotein, 5'deiodinase type I, is a major enzyme that catalyzes the deiodination process where Tyroxine (T4) is converted to Triiodothyronine (T3), an active

form of thyroid gland (6).

Another selenoprotein present in the human body is called selenoprotein P. The function is not known, but since other selenoproteins have a reduction-oxidation function, selenoprotein P might also has the same property. One hypothesis suggests that it has an antioxidant function like vitamin E.

The role of selenium in pancreatic function was mentioned in several studies where selenium deficiency is related to fibrotic degeneration of the pancreas in the chick (9,10,11,12). Table II will list the influences selenium has on pancreatic function. Two studies in particular mentioned the effect of selenium compounds on cancer cells. In 1966, Shamberger and Rudolph (13) found that topical selenite retarded the appearance of tumors more effectively compared to vitamin E or other kinds of antioxidants. Another study done by Weisberger and Suhrland in 1956 showed a remission in acute leukemia and chronic myeloid leukemia after the patients received selenocystine (14,15).

Selenium is also associated with immunity. In two studies done by Spallholz et al in 1973 and 1975 (16,17) injection of selenium as selenite and selenium and vitamin E as Seletoc® (5 μg Se/mouse) enhanced primary as well as secondary anti-sheep red blood cell (SRBC) IgM and IgG antibody titers. The group of mice fed a diet containing selenium as selenite 1-3 ppm had higher antibody titers.

Selenium, as a component of glutathione peroxidase, may also have the ability to protect membrane from damage caused by lipid peroxide-induced cellular damage by destroying that peroxide by catalyzing the reduction of lipid hyperoxides to the corresponding alcohol derivatives (18).

A study by Wu et al. in 1973 (19) showed that a female rat fed with a selenium-deficient diet gave birth to male rats that had a very few spermatozoa recoverable from cauda epidedymes. The motility of the spermatozoa was very poor and there was a breakage between the body of the spermatozoa and the tail. In this case, vitamin E or other antioxidants cannot replace selenium in solving the problem. Brown and Burke (1973) showed that selenium is concentrated in the mid-piece of spermatozoa (20). A

Table II. Influence of Selenium on Pancreatic Function (a).

	Se adequate	Se deficient
Body weight, g	294	229 (b)
Bile weight, mg	169	473 (b)
Plasma glutamic-oxaloacetic transaminase mg/ml	240	772 (b)
Plasma fat-soluble reducing substances mg/ml	23	1.2 (b)
Pancreas weight, mg/100 gr body weight	323	206 (b)
Pancreas lipase (c)	283	115 (b)
Pancreas trypsin (c)	69	34.5 (b)
Pancreas chymotrypsin (c)	235	167 (b)

a From Thompson and Scott (1970).
b Significance difference, $P < 0.05$.
c Enzyme units/100 gr body weight.

selenoprotein plays a major role in keratinized process in sperm mid-piece.

Deficiency in Human and Animal

As we start to understand the important roles selenium has in the human body, it is obvious that the greatest health risk of selenium deficiency is present among infants and young children.

In late 1970s, a study in one province in China reported two human diseases associated with selenium deficiency (21). Those diseases are juvenile cardiomyopathy (used to be called Keshan disease), and chondrodystrophy named by its founder Kaschinbeck disease. The affected areas have a very low selenium concentration in their soil which leads to an extremely low selenium concentration in their foods.

Keshan disease is a multifocal inflammation of heart muscle that occurs primarily in children aged 2 to 10 years old, and in some women of child-bearing age. The diagnosis

of this disease is based on acute and chronic insufficient cardiac function, cardiomegaly, and abnormalities in electrocardiogram and radiogram. Some patients show cardiogenic shock or congestive heart failure and embolic episodes from cardiac thromboses. The heart is the major organ that is affected by the disease, but some patients may also have hepatic congestion, mesenteric lymphadenosis, degenerative diaphragm changes, and pancreatic exocrine dysfunction. When infants start eating solid food, their selenium intake decrease subtantially due to an extremely low selenium content in food items. This is the time when the first sign of deficiency is observed. The severity of the disease can be reduced by giving the patients oral selenium supplementation.

Kaschin-Beck disease is an osteoarthropathy that affects primarily the epiphyseal and articular cartilage and the epiphyseal growth plates of the growing bones. The affected cartilage shows atrophy and necrosis with repair and endochondral ossification. The most striking condition is chondronecrosis with proliferation of surviving chondrocytes in clusters. This condition leads to enlargement of the joints (fingers, toes, elbows, and knees); shortened fingers, toes, and extremities. Dwarfism is the result in severe cases. The prevalence of the disease is greatest among children aged 6 to 15 years old. Since the relation between this disease and selenium deficiency is less certain compared to Keshan disease, limited studies suggest that oral selenium supplementation may be an effective way to cure the disease. A more rigorous testing is needed.

A combination of selenium deficiency and vitamin E deficiency causes liver necrosis in rats and swine, exudative diathesis in chickens, and in sheep and cattle white muscle disease. A study done by McCoy and Weswig in 1969 (22), showed that animals fed with a selenium-deficient diet and adequate vitamin E had hair loss, growth retardation, and reproductive failure. Another study by Thompson and Scott in 1970 (23) showed pancreatic degeneration in chicks with a very severe selenium deficiency.

Toxicity in Human and Animals

In livestock, selenium toxicity will happen if the intake exceeds 4 to 5 μg/g. Chronic selenosis is characterized by cirrhosis, lameness, hoof malformations, hair loss,

and emaciation (24). The most common signs of selenium intoxication are hair loss and nails. In some areas, lesion of the skin, abnormal nervous system and teeth condition were also observed.

Requirement and Recommended Intake

The National Research Council established an estimated safe and adequate daily dietary intake of selenium for adult in 1980 to be 50 to 200 μg (25). This number was extrapolated from animal experiments due to very few data available from human experiments. A survey was done and showed that Keshan disease was absent in the area where the selenium intake was at least 19 μg in male and 13 μg in female (21). This can be considered to be the minimum amount of intake for human. According to Levander (26) plasma glutathione peroxidase activity is maximized in individuals who received an additional 30 μg of selenium to the 11 μg of selenium from a daily habitual intake. The total amount for a maximum glutathione peroxidase activity is then 41 μg. This number is then multiplied and added to it the safety factor to make the total amount of 70 μg for male and 55 μg for female. The recommendations for children were extrapolated from the adult values on the basis of body weight. The "normal" North Americans diet should fulfill this amount without any trouble at all. Special cases may arise for people who lives in a certain area which has an extraordinary low level of selenium in the soil and are unable to obtain food from other places.

The Recommended Dietary Allowance (Revised in 1989) for selenium is listed in Table III.

Table III. Recommended Dietary Allowance of Se (Revised 1989) in µg/day.

Category	Age (years)	Weight (a) lb	Height (a) in	Se µg/d
Infants	0.0 - 0.5	13	24	10
	0.5 - 1.0	20	28	15
Children	1 - 6	29 - 44	35 - 44	20
	7 - 10	62	52	30
Males	11 - 14	99	62	40
	15 - 18	145	69	50
	19 +	160 - 170	70	70
Females	11 - 14	101	62	45
	15 - 18	120	64	50
	19 +	128 - 143	65	55
Pregnant				65
Lactating				75

From Food and Nutrition Board, National Research Council: RDA 10th Ed.

Washington, D.C., National Academy Press, 1989.

Manganese

History

Manganese was found to be a constituent of animal tissue in 1913. In 1931, Orent and McCollum found that deficiency in manganese shown to induce growth retardation in mice and an abnormal reproduction function in rat (27).

Roles and Function

Manganese has two major biochemical functions that are known at the present time. Manganese is important as an enzyme activator and as a constituent of metalloenzymes. Several enzymes that need manganese for activation are hydrolases, kinases, decarboxylases, and transferases (28). Not all of these enzymes are manganese specific enzymes, some of them can be activated by other element such as magnesium. Those that are manganese specific enzymes includes glycosyltransferases and xylosyltransferases. There are only few manganese metalloenzymes, arginase, pyruvate carboxylase, glutamine synthetase, and manganese superoxide dismutase.

Pyruvate carboxylase is important in carbohydrate metabolism and that means manganese plays a major role in carbohydrate metabolism. According to White et al, (29) manganese is an activator of insulin receptor protein kinase.

In 1970, Friedman and Rasmussen reported that manganese increases gluconeogenesis from lactate in the perfused rat liver (30), but it is not clear where exactly in the pathway manganese has its affect.

Manganese is recognized to be essential for normal connective tissue, skeletal, and nervous system development (31). One most striking effect apparent following acute manganese deficiency is ataxia and audiogenic seizures (32). On the other hand, manganese toxicity leads to primarily neurological symptoms such as tremor, rigidity, and hallucinations.

Manganese also is essential for the oxidation of water to O_2 in the photosynthetic process. Manganese is required for the catalyst of the four-electron oxidation of water

within the active site of the complex: (Mn)

$$2H_2O \text{ ---------> } O_2 + 4e^- + 4H^+$$

Deficiency in Human and Animal

In many species of animals, deficiency in manganese causes impaired growth, skeletal abnormalities, depress reproduction function, ataxia in newborn, and defects in lipid and carbohydrate metabolism (28). But in humans, deficiency in manganese is less conclusive.

Toxicity in Human and Animal

Again in animals, overdoses of manganese leads to depressed growth, depressed appetite, impaired iron metabolism, and abnormal brain function (28).

In man incidents of manganese toxicity are normally the result of chronic inhalation of large amount of airborne manganese, and not from the diet. But it is not until 1930's and 1940's that manganese toxicity was recognized as a potentially serious to human health (33). In individual who are exposed to high manganese environment, the sign of toxicity occur only after few months or few years. It is characterized by severe psychiatric disorder resembling schizophrenia progressing to crippling neurological disorder similar to Parkinson's disease.

Manganese toxicity altered carbohydrate metabolism. Rubenstein et al (34) suggested a possible relationship between manganese and carbohydrate metabolism, where an insulin resistant diabetic patient responded to oral doses of $MnCl_2$ and decrease the blood glucose level.

Requirement and Recommended Intake

There is no RDA for manganese, instead the estimated safe and adequate daily dietary intake (ESADDI) is used as a guideline (Table IV). The value of ESADDI were set mainly because most food manganese content fall within this range without producing either deficiency or toxicity.

Table IV. The Estimated Safe and Adequate Daily Dietary Intake for Mn.

Category	Age (years)	Mn (mg/day)
Infants	0 - 0.5	0.3 - 0.6
	0.5 - 1	0.6 - 1.0
Children	1 - 3	1.0 - 1.5
	4 - 6	1.5 - 2.0
	7 - 10	2.0 - 3.0
	11 - older	2.0 - 5.0
Adult		2.0 - 5.0

From Food and Nutrition Board, National Research Council: RDA 10th Ed. Washington, D.C., National Academy Press, 1989.

Zinc

History

In 1934, Todd et al (35) reported the essentiality of zinc for growth and well being in rats for the first time. Tucker and Salmon (36) reported that zinc cures and prevents parakeratosis in swine. Zinc deficiency in man was suspected to occur for the first time in 1961 in Iranian males (37).

Roles and Function

Growth retardation in rats mentioned in Todd et al. study (35) was suggested to be caused by a decrease activity of thymidine kinase that leads to impaired DNA synthesis and cell division. In different study done by Mills et al.(38), lambs and calves given a severely zinc-deficient diet ceased growth abruptly and within two weeks, growth stop. Pregnant rats given a zinc-deficient diet had an impaired fetal growth while such a diet fed during lactation impairs growth in suckling pups (39). Zinc deficiency in male human manifested in dwarfism and hypogonadism. These characteristics first were studied by Prasad in Iran and Egypt in 1960's (37). As soon as adequate zinc was provided, stimulation to growth is resumed.

Growth retardation is also partly resulted from impaired appetite. The study done by Chesters and Quarterman (40) showed that voluntary food intake of zinc-deficient rats decrease up to 70% compare to control. When the rats were fed with zinc-supplemented diet, normal eating pattern was obtained within two hours. Henkin and associates (41) were the first group that establish the physiological role of zinc in normal taste sensation. Further study showed that hypogeusia or loss of taste acuity and dysgeusia or disorder taste can respond rapidly to zinc therapy.

Rats and mice with zinc deficient condition also suffer from alopecia and gross skin lesions. Parakeratosis of epithelial cells and esophagus was also disclosed in histological studies (42). In more severe condition, loss of hair, dermatitis, scaling and cracking of the paws with deep fissures develop (43). The healing of all these conditions are very rapid and dramatic. Acrodermatitis enteropathica, a hereditary disease caused by an abnormal

zinc metabolism, responds very well to zinc supplementation.

Zinc deficiency also has a very detrimental effect on reproduction. The development of the primary and secondary sex organ in male and spermatogenesis, and all the reproductive phases in female affected by zinc deficiency. Retarded development of testes, epididymis, prostate, and pituitary gland was reported along with atrophy of the testicular germinal of epithelium by Mawson et al.(44) In part of Iran and Egypt, hypogonadism with the suppression of secondary sex organs were observed in young male and young female (37).

Several species of bird show an abnormal skeletal development with zinc deficiency (45). The long bones are shortened and thickened and the severity if proportional to the severity of the deficiency. Supplementation of zinc clearly prevent this condition although the nature of interaction remain obscure. In chick, osteoblastic activity is decrease in the long bones, together with a decrease in chondrogenesis in relation with an increase in the amount of cartilage matrix (46).

The role of zinc in wound healing have produced conflicting results. This role was first observed by Pories and associates (47) in the study where a young man, following a surgery forpilonoidal sinuses, was given zinc sulfate (50 mg of zinc) three times a day. His rate of wound healing was much faster compare to unmedicated patient. Several other patients with severe burns and major surgery demonstrated improved wound healing. This increase in speed of wound healing causes by zinc may be explained in the fact that the metabolic demand for this element in collagen synthesis in the process of tissue repair is increased (48), but the direct evidence is lacking.

There has been an indication that zinc may have a beneficial effect in some cases of atherosclerosis. Pories et al.(47) gave zinc sulfate to 13 patients with an advance vascular disease for 29 months. Twelve of them showed a marked improvement and nine return to a normal activity. The mode of action of zinc in atherosclerosis is unknown, but since atherosclerosis is thought to start with some form of trauma it may be, in part, an expression of inadequate arterial repair.

The beginning of fifth week of pregnancy is a critical period for brain growth. Zinc deficiency during this sensitive time will permanently affects brain function. When this deficiency is continue throughout the latter third semester of pregnancy, the brain size will decrease. There is a reduce in total brain cell number and the cytoplasmic nuclear ratio is increased which signs an impairment of cell division in the brain during macroneuronal proliferation. Zinc deficient suckling rats have retarded brain maturation indicated with a reduce total cerebellar lipid concentration, and a significantly lower rate of protein synthesis in the brain (50). The same study showed that a delay in myelination is also found in rat pups when zinc is deprived during gestational and lactation period that leads to a significant reduction in rat brain 2',3'-cyclic nucleotide 3'-phosphohydrolase.

Zinc is involved in nucleic acid and protein metabolism which means it is important in cell replication process. Zinc deficiency leads to an impairment of DNA synthesis in the liver (50) and reduce total protein and RNA contents of testes. Utilization of amino acids in the synthesis process of protein impaired in zinc deficiency (51).

Prasad and associates (37) found in their study that several enzymes decrease their activities when zinc level is reduced. In testes, lactic dehydrogenase (LDH), malic dehydrogenase (MDH), alcohol dehydrogenase (ADH), and NADH diaphorase; in the bone, LDH, MDH, ADH, and alkaline phosphatase; in esophagus, MDH, ADH, and NADH diaphorase; and in the kidney, MDH and alkaline phosphatase were decreased. Pancreatic carboxypeptidase activity was also found reduced in zinc deficient rats and return to normal rapidly with zinc therapy.

Deficiency in Human and Animal

The growth retardation found in zinc deficiency does not seems to be caused only by decreased food intake. Study by Miller et al.(52) showed that young pigs given zinc-deficient diet declined in their body weight even before food intake was affected. Nutrients utilization was impaired perhaps due to changes in enzyme activities caused by zinc deficiency. Paired-feeding in rats also showed a similar result. The same amount of food was given to both group, but the group given a zinc-deficient diet had significantly lower

body weight compare to the other group.

Zinc was found in small amount in crystalline insulin. When glucose was injected intraperitoneally into rats with a low zinc-deficient diet for a longer period of time, their glucose tolerance of these rats was depressed (53). It is believed that the rate of insulin secretion in response to glucose ingestion is decreased in zinc deficiency.

The effect of zinc deficiency in growth and sex hormones was observed in in-vitro studies with human cell culture. Injection of gonadotropin and testosteron, under zinc deficiency condition, promote male accessory sex organs but did not prevent testes tubular atrophy (54).

Zinc has been shown to be a structural component of several metalloenzymes and plays a functional role in some of them. In the case of deficiency, it is suspected that the activity of those enzymes will change. The study done by Guttikar et al.(55) showed that serum alkaline phosphatase level is not affected by calorie intake, on the other hand its activity loss is directly attributed to zinc deficiency. Other enzymes such as pancreatic carboxipeptidases, which are important in protein digestion, loss its activity in zinc deficiency. Prasad and Oberleas (56) has done some studies to see if zinc status will change the activity of alcohol dehydrogenase. The studies showed clearly that zinc deficiency lowers the activity of this enzyme in the liver, bones, testis, kidneys, and esophagus in rats and pigs. Zinc deficiency also may affect RNA and DNA metabolism in organisms and plants which then responsible for impaired protein synthesis (57).

Toxicity in Human and Animal

Three studies in late 1960 and early 1970 suggested three types of zinc toxicity in human. First type of toxicity, *metal fume fever*, was observed in some industrial workers who are exposed to the fumes, and the symptoms are fever and gastroenteritis. The second type was observed in a 16-year old boy who ingested 12 mg of Zinc sulfate within 2 days, and the symptoms are lethargy, increased serum lipase and amylase levels (58). The third type has been observed in a patient with renal failure following hemodialysis (59), and the symptoms are nausea, vomiting, fever, and severe anemia.

An animal study was done by Van Reen (60) showed that rats ingested 0.5-1.0% zinc have a reduced growth, anemia, poor reproduction, and a decreased activity of liver catalase and cytochrome oxidase. This condition can be reversed by supplementing the rats with copper. This suggest that zinc toxicity may cause copper deficiency.

Requirement and Recommended Intake

The 1989 RDA for zinc are based on the intakes required to maintain balance and to replace endogenous losses. The revised RDA is listed in Table V.

Table V. Recommended Dietary Allowance for Zn (revised 1989) in mg/day.

Category	Age (years)	Males	Females
Infants	0.0 - 0.5	5	5
	0.5 - 1.0	5	5
Children	1 - 3	10	10
	4 - 6	10	10
	7 - 10	10	10
Adolescents	11 - 18	15	12
Adults	19 - 51+	15	12
Pregnancy			15
Lactation	First 6 months		19
	Second 6 months		16

From Food and Nutrition Board, National Research Council: RDA 10th Ed. Washington, D.C., National Academy Press, 1989.

Copper

History

Copper was therapeutically used around 400 BC. It was prescribed for pulmonary and other diseases (61). The thought that copper is essential to human body was not exist, not until the second decade of the century following the discovery of several vitamins. In 1928 an animal study showed a certain kind of anemia that could not be prevented by iron supplement only. This anemia was responsive to iron only if copper supplement was also given at the same time. Copper was link to human disease for the first time around 1912, when Wilson's disease was described, but a conclusive evidence of copper deficiency was not reported until 1964.

Roles and Functions

Copper is an important part of many enzymes and proteins (62). Several copper-containing enzymes found in human beings are: monoamine oxidase (involved in inactivation of catecholamines), diamine oxidase (inactivates histamine, polyamines), lysyl oxidase (play role in the formation of connective tissue), ceruloplasmin (catalyzes the oxidation of ferrous iron, transfer iron from storage site for hemoglobin synthesis), ferroxidase II (catalyzes the oxidation of ferrous iron), cytochrome c oxidase (reduces oxygen to form water), dopamine ß-hydroxylase (catalyzes the convertion of dopamine to norepinephrine), extracellular superoxide dismutase (scavenges superoxide radicals and protect against oxidative damage), copper/zinc superoxide dismutase (protect intracellular from oxidative damage), tyrosinase (catalyzes the convertion of tyrosine to dopamine and the oxidation of dopamine to dopaquinone, also required for melanin synthesis).

Some physiological functions of copper are also well known (61). Copper,through lysyl oxidase, plays an important role in collagen and elastin cross-linking. Collagen and elastin are required to make a strong yet flexible connective tissue. Copper, as a component of ceruloplasmin and ferroxidase II, is essential in the formation of bone marrow cells necessary for red blood cell synthesis. In central nervous system, copper is

required for the formation and maintenance of myelin sheet.

Deficiency in Human and Animal

Copper deficiency has been observed particularly in human infants (61). Premature infants, infants 6 to 18 months, or those recovering from marasmus on exclusive milk diet are susceptible to copper deficiency. The major manifestation of copper deficiency are: neutropenia, hypochromic anemia, osteoporosis with the enlargement of costochondral cartilages (scurvy-like condition), decrease pigmentation of the skin, chronic diarrhea, and in later stages, neurological abnormalities such as hypotonia, apnea, and psychomotor retardation.

Toxicity in Human and Animal

Oral ingestion of excess copper causes a metallic taste in the mouth, nausea vomiting, epigastric pain, diarrhea, a variable degrees of jaundice, hemolysis, hemoglobinuria, hematuria, and oliguria. In some severe cases, anuria, hepatic necrosis, vascular collapse, hypotension, coma, and death can occur (61). Hemolysis, due to an excess copper in red blood cells, may inhibit erythrocyte glycolysis, glucose-6-phosphate dehydrogenase, oxidation of glutathione, and denaturation of hemoglobin.

Copper toxicity in young infants, under normal circumstances, has been reported, presumably exposed via drinking water, cooked food, and contamination from all-copper containers (61). Renal patients undergo dialysis are also prone to copper toxicity.

Requirement and Recommended Intake

Mason (61), in his article, defined the term of minimal intake as the daily intake which equals the daily excretion. To know exactly how much copper is needed by human body is difficult to establish due to several factors such as the variability of the amount actually being absorbed by the body, slow rate of turnover, and exclusive output via the feces. But, nevertheless, a guideline has been established by the National Research Council of the United States concerning copper requirement intake. The Estimated Safe and Adequate Daily Dietary Intake for cooper is listed in table VI.

Table VI. The Estimated Safe and Adequate Daily Dietary Intake for Cu in mg/day.

Category	Age (years)	Amount (mg/d)
Infants	0.0 - 0.5	0.4 - 0.6
	0.6 - 1.0	0.6 - 0.7
Children	1 - 3	0.7 - 1.0
	4 - 6	1.0 - 1.5
	7 - 10	1.0 - 2.0
	11+	1.5 - 2.5
Adults		1.5 - 3.0

From Food and Nutrition Board, National Research Council: RDA 10th Ed. Washington D.C., National Academy Press, 1989.

CHAPTER III

METHODS AND PROCEDURES

Selenium, manganese, zinc and copper content of sixteen different commercially prepared infant formulas were determined. The sixteen formulas represent six basic formula names, but these six have different major constituent sources (soy protein and cow milk), they differ within brand names according to iron content (low iron or added iron), form (ready-to-feed, concentrated or powdered), and formula storage environment (metal cans or glass bottles). Details are listed in Appendix A.

This chapter describes the selection of infant formulas, labeling the formula to be used, preparation for analysis, sampling, wet ashing, and atomic absorption spectrophotometry. The data were then statistically analyzed.

Labeling The Infant Formula to be Used

Random Selection From Each Lot

From each lot for each formula, two or three containers were randomly selected. They were, then, labeled by letter chronologically, A through P, according to their expiration date to insure that no analysis would represent an outdated formula. Since two or three containers were taken from each lot, additional secondary numbers representing these, accompanied each letter, for example A1, A2, A3, B1, and so on. Thus, after samples were taken, there was no direct identification of letter assigned to a particular formula. Appendix B gives a listing of formulas and detailed information such as lot number, expiration date of the lot, and number of containers provided for random selection for analyses.

Preparation For Analyses

Sampling Procedure

From each can or bottle, four samples were taken for mineral analyses. Since two or three cans or bottles (depending on numbers in the lot; see Appendix II) were taken from each of the 16 lots to make the total of 40 cans or bottles, the total samples taken were 160.

For mineral analyses, regular glass test tubes were used to hold the samples.

* Three hundreds of these glass test tube were acid washed for at least 24 hours to make sure that all the test tubes were clean from any kind of minerals that can and will distorted the result of the mineral analyses.
* After being soaked for at least 24 hours in acid bath, the tubes were then washed with distilled water to rinse off all the acid. Distilled water from the same source was used in preparation of the infant formulas.
* All the test tubes, after they were rinsed, were put in the oven at 100°C for one day.
* The mouth of the test tubes were then covered with parafilm (Parafilm "M"® laboratory film; American National Can; Greenwich, CT. 06836) to avoid contamination.
* The test tubes were marked with ceramic marker and let dry for 10 to 15 minutes.
* The test tubes were put upside down inside a 500 mL acid washed beaker covered by acid washed petri dish.
* Again the beakers were put in the oven over night at 100°C.
* The test tubes were taken out the next day and let cool down at room temperature.
* The test tubes were weighed.

When all the test tubes were ready, the sampling began.

* A 1000 µL micropipette was used to take the sample from the can or bottle.

* All sampling process was done under the hood to avoid contamination.
* The container of all liquid formula were thoroughly shaken to distribute the precipitation that occured during storage.
* The tops of the containers were rinsed with distilled water and wiped dry with Kim Wipe.
* If the particular infant formula is a ready to feed formula, no distilled water was added.
* For concentrated formula, 10 mL of the formula was diluted with distilled water according to the manufacture to be ready to use.
* For powdered formula, one spoon , using the spoon provided with the formula which was inside the container, was used and diluted with distilled water according to the manufacturer to be ready-to-used.
* Four samples were taken from each of the formulas using a 1000 μL micropipette, placed in the prepared test tube, and covered with parafilm to avoid contamination.
* After all the sampling was done, the test tubes, again, were covered with parafilm to avoid contamination.

All sampling procedures except for rinsing of the container top were carried under the hood.

Ashing Procedure

The procedure used in the study was based on the procedure developed by Litov (21).

* Since the space in heating block for wet ashing was limited (96 test tubes at one time), the samples were divided into two groups. While the first half was in the heating block, the second half was stored in the freezer.
* One empty acid-washed tube containing distilled water instead of sample

was placed in the heating block with each run to serve as the control and was treated identically to the samples (see Appendix C), and treated the same way as other tubes containing the sample.

* Distilled water (the same distilled water used during the previous steps), nitric acid (Lot # R17), and hydrogen peroxide (Lot # C40338) were used for wet ashing.
* 500 µL of nitric acid was added to each test tube.
* The test tubes were then put in the heating block at 80°C overnight.

Appendix A shows the configuration of the test and control tubes in the heating block during wet ashing.

* The following day :

    a. The temperature was increased to 100°C

    b. 250 µL of hydrogen peroxide after all but about 1 mL of solution had evaporated from all tubes.

    c. 100 µL of hydrogen peroxide was added every two hours for one week until the dry crystal left in the tube was pure white in color.

    If one of the tubes (either test tube or control tube) was dry the next day, 100 µL of distilled water, nitric acid, and hydrogen peroxide were added to all of the tubes, including the one that was not dry. Then, 50 µL of hydrogen peroxide was added every two hours instead of 100 µL.
* When all the tubes were ready, the heating block was turn off.
* All the tubes were, then, again stored inside an acid washed beaker and petri dish and stored inside a plastic container.
* The second half was the put in the same heating block with the same treatment as the first half.

* After all the samples cooled to room temperature, they were weighed again to determine the dry sample weight after wet ashing. They were all listed in Appendix D.

Acidity Measurement

The acidity of each infant formula was measured with Fisher Accumet® Model 815MP pH Meter immediately after all the sampling was done. The test was done directly in the original can or bottle of each infant formula at the same room temperature of 26° C. The results were also listed in Appendix D.

Atomic Absorption Procedure

* Four minerals were quantified with the Atomic Absorption Spectrophotometer (Perkin-Elmer 5100PC GFAAS - Zeman). Zinc and copper were measured on the flame side and manganese and selenium on furnace side.
* All the dry samples in glass tubes were diluted with 200 µL of concentrated nitric acid and 1.3 mL of distilled water. Since the volume of original sample was 1000 µL and now it was diluted to 1500 µL, the original dilution factor (df) is, then, 1.5. The mixture was then vortexed vigorously before it was poured to a sterile polyetheline tube (Falcon® Tool of Discovery; Becton Dickinson Labware; Lincoln Park, NJ. 07035) before being stored in the refrigerator.
* For copper and zinc, the original dilution factor was used for the analyses.
* For manganese: 50 µL of original sample was added with 3.0 mL of 0.5% of nitric acid.

  df = [(50 µL + 3.0 mL) : 50 µL] x 1.5 = 91.5
* For Selenium:

  Two out of four samples from each can or bottle with the closest dry weight

value were chosen.

500 µL original solution (df=1.5) was added with 750 µL of 0.5% nitric acid. The new df=3.75.

For each tube, four new sterile polyetheline tubes were labeled (e.a.: 23-1, 23-2, 23-3, 23-4).

On all tubes labeled -1, 250 µL of 0.5% nitric acid was added.

On all tubes labeled -2, 250 µL of 50 ppb selenium standard was added.

On all tubes labeled -3, 250 µL of 100 ppb selenium standard was added.

On all tubes labeled -4, 250 µL of 150 ppb selenium standard was added.

* The result of the analysis can be found in Appendix E.

During all the preparation for analysis, hair net, laboratory coat, and mineral-free gloves were used at all time to prevent contamination.

Statistical Analysis

A simple descriptive statistic analysis is used (mean, standard deviation, and range) to analyze the data in this study. Limited number of samples from different lots prevented any further analysis such as student t-test analysis.

CHAPTER IV

RESULTS AND DISCUSSION

Trace Minerals Content of Infant Formula Compared to Breast Milk

Data were analyzed to determine zinc, copper, manganese, and selenium content in various infant formulas. The mean concentrations of zinc and copper in various infant formulas were determined for comparison with values reported by the manufacturer and for comparison with the concentrations in breast milk reported by the Committee on Nutrition of the American Academic of Pediatrics 1985 (2). The mean concentrations of manganese and selenium in various infant formulas were determined for comparison with thevalue in breast milk reported by the Committee on Nutrition of the American Academic of Pediatrics 1985.

Tables VII to X summarize the mean concentrations of zinc, copper, manganese, and selenium from the infant formulas. The standard deviations from the mean (SD) and the highest and lowest values from each infant formula are also shown along with the actual concentrations range of zinc reported by the manufacturer and the concentrations in breast milk ($\pm$SD). The ratio between the value from the experiment and from breast milk is shown in figure I to IV.

After comparison was made between the content of zinc in various infant formulas being tested and the content of zinc in breast milk, it was clear that the zinc content of infant formula was 4.2 to 8.5 times higher than that was in breast milk. Infant formula being tested contained 2.2 to 6.9 times more copper than breast milk. The infant formulas contained a wide range og manganese. It ranged from 8.2 to 55.00 times that of breast milk. For selenium, infant formulas contained 1.8 to 3.3 times more selenium than breast milk.

Zinc and copper content of infant formulas made from cow milk and soy bean were similar. For manganese, infant formulas made from soy bean consistently had a higher

concentration when compared to those made from cow milk. Computer analysis showed that 1 cup (186 g) of dried soy bean contains 4.69 mg of manganese while 1 cup (244 g) of cow milk contains only 0.01 mg. For selenium, the differences between infant formulas made from soy bean and cow milk were not as great, but still consistantly higher in those made from soy bean. Computer analysis again showed that 1 cup (186 g) dried soy bean contains 10.6 μg of selenium while 1 cup (244 g) of cow milk contains only 3.0 μg.

Trace Minerals Intake from Infant Formula Tested Compared to the Latest Recommendations

Table XI to XIV again summarize the mean value of zinc, manganese, copper, and selenium ± SD from the analysis, the latest recommendation for each mineral (RDA and ESADDI) for age group 0 - 1 year old, the infant average intake of each mineral in one day when the infant consumes only formula and not breast milk. The ratio between the recommendation and the intake is shown in figure V to VIII.

For zinc, the Recommended Dietary Allowance (1989) for age group 0 - 1 year old is 5 mg/day. According to Fomon SJ, et al. (1), average infants consume 16 to 32 fl.oz. of milk in one day. When those numbers were used to calculate the intake of mineral from infant formula being tested, on a daily basis infants get between 0.47 to 1.93 times the RDA for zinc from infant formula.

For copper, the Estimated Safe and Adequate Daily Dietary Intake for age group 0 - 1 year old is between 0.4 to 0.6 mg/day. Again when the results were calculated using the average amount of milk consumed in one day, infants would get between 0.43 to 2.7 times of the ESADDI for copper from the infant formulas being tested.

For manganese, the Estimated Safe and Adequate Daily Dietary Intake for age group 0 - 1 year old is between 0.3 to 1.0 μg/day. The ratio between the intake of manganese from infant formulas being tested ranged between 0.02 to 0.31 times the ESADDI for manganese.

For selenium, the Recommended Dietary Allowance (1989) for age group 0 - 1 year

old is 10 to 15 μg/day. The ratio between the intake of selenium from infant formula being tested is ranged between 1.12 to 4.15 times he RDA for selenium.

Since there were not enough sample available for the experiment, the significant of the results of the experiment cannot be determined. The result reported above should be used to guide further study towards any of these trace minerals to see if they are consistently high in all infant formulas available in the market and not only in certain lots. An absorption study then can be done to see the significance of this different level of trace minerals in infant formula compared to breast milk to see if the excess of trace minerals in infant formula is all being absorbed or not.

Table VII. Zinc Concentration of Prepared infant Formulas: Analyzed and Manufactured.

Sample	Analyzed			Printed on Label (c)
	n	Concentration (a)	Range (b)	
ALIMENTUM®-Protein Hydrolysate w/Iron (ready)	12	5.12±0.45	3.79-5.57	5.07
SIMILAC®PM60/40-Low iron (ready)	11	5.63±0.30	5.08-5.98	5.07
SIMILAC®-Low Iron (ready)	12	5.27±0.46	4.39-5.86	5.07
ISOMIL®-Soy formula w/Iron (ready)	11	5.26±0.32	4.50-5.76	5.07
SIMILAC®-Special Care w/Iron (ready)	12	10.20±0.69	8.66-10.96	10.14
SIMILAC®-w/Iron (concentrated)	10	5.28±0.21	4.76-5.47	5.07
SIMILAC®-Low Iron (conc.)	12	5.13±0.92	2.17-5.81	5.07
SIMILAC®-w/Iron (ready)	12	5.08±0.46	3.78-5.44	5.07
ISOMIL®-Soy formula w/Iron (concentrated)	8	5.23±0.39	4.50-5.85	5.07
ISOMIL®-Soy formula w/Iron (ready)	8	5.46±0.31	4.80-5.85	5.07
SIMILAC®-w/Iron (ready)	8	5.27±0.68	3.84-5.84	5.07
SIMILAC®-w/Iron (ready)	8	5.30±0.19	4.80-5.39	5.07
SIMILAC®-Low Iron (powder)	8	5.19±0.09	5.02-5.32	5.07
SIMILAC®-w/Iron (powder)	8	5.15±0.55	3.76-5.56	5.07
SIMILAC®PM60/40-Low Iron (ready)	8	5.23±0.33	4.82-5.69	5.07
ISOMIL®-Soy formula w/Iron (powder)	8	5.05±0.28	4.62-5.39	5.07

Zinc concentration of breast milk = 1.2 ± 0.2 mg/l (2).

a Mean ± SD in mg/l.

b Range in mg/l.

Table VIII. Copper Concentration of Prepared infant Formulas: Analyzed and Manufactured.

Sample	Analyzed			Printed on Label (c)
	n	Concentration (a)	Range (b)	
ALIMENTUM®-Protein Hydrolysate w/Iron (ready)	12	0.63±0.04	0.50-0.68	0.51
SIMILAC®PM60/40-Low iron (ready)	11	0.62±0.03	0.57-0.66	0.61
SIMILAC®-Low Iron (ready)	12	0.62±0.03	0.56-0.65	0.61
ISOMIL®-Soy formula w/Iron (ready)	11	0.58±0.14	0.29-0.64	0.51
SIMILAC®-Special Care w/Iron (ready)	12	1.73±0.13	1.38-1.84	1.69
SIMILAC®-w/Iron (concentrated)	10	0.61±0.16	0.20-0.76	0.61
SIMILAC®-Low Iron (conc.)	12	0.63±0.08	0.41-0.80	0.61
SIMILAC®-w/Iron (ready)	12	0.61±0.04	0.54-0.66	0.61
ISOMIL®-Soy formula w/Iron (concentrated)	8	0.56±0.09	0.34-0.63	0.51
ISOMIL®-Soy formula w/Iron (ready)	8	0.53±0.03	0.54-0.63	0.51
SIMILAC®-w/Iron (ready)	8	0.62±0.11	0.34-0.66	0.61
SIMILAC®-w/Iron (ready)	8	0.60±0.04	0.56-0.66	0.61
SIMILAC®-Low Iron (powder)	8	0.66±0.05	0.59-0.72	0.61
SIMILAC®-w/Iron (powder)	8	0.63±0.03	0.59-0.67	0.61
SIMILAC®PM60/40-Low Iron (ready)	8	0.64±0.21	0.22-0.82	0.61
ISOMIL®-Soy formula w/Iron (powder)	8	0.54±0.02	0.53-0.58	0.51

Breast milk copper concentration = 0.25 ± 0.03 mg/l (2).

a Mean ± SD in mg/l.

b Range in mg/l.

Table IX. Manganese Concentration of Prepared Infant Formulas: Analyzed.

Sample	n	Concentration (a)	Range (b)
ALIMENTUM®-Protein Hydrolysate w/Iron (ready)	11	245±9.84	235-268
SIMILAC®PM60/40-Low iron (ready)	10	48.91±4.58	43-57
SIMILAC®-Low Iron (ready)	10	81.92±7.74	78-96
ISOMIL®-Soy formula w/Iron (ready)	11	225±39.70	196-282
SIMILAC®-Special Care w/Iron (ready)	12	70±31.50	124-214
SIMILAC®-w/Iron (concentrated)	11	65.73±5.24	56-70
SIMILAC®-Low Iron (concentrated)	12	52.25±2.75	48-58
SIMILAC®-w/Iron (ready)	10	55.50±10.74	45-80
ISOMIL®-Soy formula w/Iron (conc.)	6	295±13.34	266-305
ISOMIL®-Soy formula w/Iron (ready)	8	304.88±4.01	299-313
SIMILAC®-w/Iron (ready)	8	62.63±4.59	53-74
SIMILAC®-w/Iron (ready)	8	61.25±6.28	49-68
SIMILAC®-Low Iron (powder)	6	60.50±11.21	46-81
SIMILAC®-w/Iron (powder)	8	66.13±9.17	55-89
SIMILAC®PM60/40-Low Iron (ready)	8	69.00±5.83	62-78
ISOMIL®-Soy formula w/Iron (powder)	8	329.88±6.94	321-343

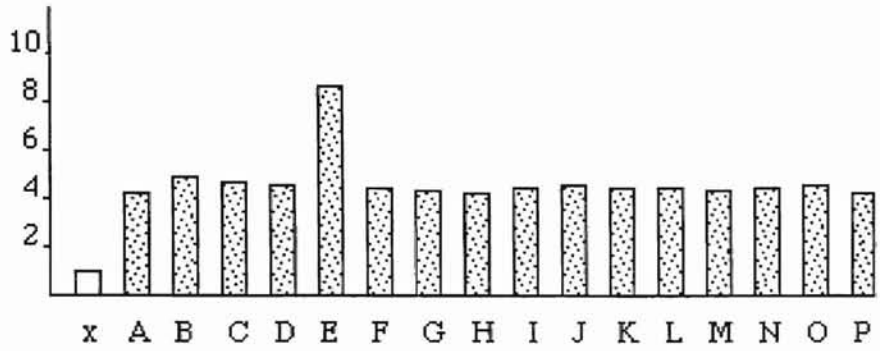
Manganese content of standard milk from National Bureau of Standards Certificate of Analysis = 260 ± 60 µg/l and from the test = 277 ± 52 µg/l.
Breast milk manganese concentration = 6 ± 2 µg/l (2).
a Mean ± SD in µg/l.
b Range in µg/l.

Table X. Selenium Concentration of Prepared Infant Formulas: Analyzed.

Sample	n	Concentration (a)	Range (b)
ALIMENTUM®-Protein Hydrolysate w/Iron (ready)	6	35.33±3.40	30 - 40
SIMILAC®PM60/40-Low iron (ready)	6	41.67±2.21	39 - 45
SIMILAC®-Low Iron (ready)	6	50.67±3.68	45 - 57
ISOMIL®-Soy formula w/Iron (ready)	6	53.83±2.12	50 - 57
SIMILAC®-Special Care w/Iron (ready)	6	59.00±3.92	53 - 64
SIMILAC®-w/Iron (concentrated)	6	46.33±2.36	44 - 50
SIMILAC®-Low Iron (concentrated)	6	45.83±2.40	42 - 49
SIMILAC®-w/Iron (ready)	6	48.50±2.57	45 - 51
ISOMIL®-Soy formula w/Iron (conc.)	4	63.50±2.29	60 - 66
ISOMIL®-Soy formula w/Iron (ready)	4	63.50±2.25	62 - 67
SIMILAC®-w/Iron (ready)	4	55.50±3.57	51 - 59
SIMILAC®-w/Iron (ready)	4	62.00±4.64	55 - 68
SIMILAC®-Low Iron (powder)	4	55.75±2.68	53 - 60
SIMILAC®-w/Iron (powder)	4	64.00±1.23	62 - 65
SIMILAC®PM60/40-Low Iron (ready)	4	66.00±4.64	61 - 72
ISOMIL®-Soy formula w/Iron (powder)	4	50.00±4.12	47 - 57

Selenium content of standard milk from National Bureau of Standards Certificate of Analysis = 110 ± 10 µg/l and from the test = 102 ± 15 µg/l.
Breast milk selenium concentration = 20 ± 5 µg/l (2).
a Mean ± SD in µg/l.
b Range in µg/l.

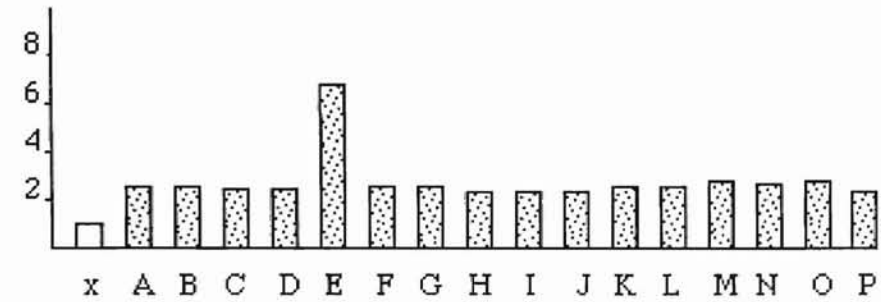
Figure I. The Ratio Between Zinc Content in Infant Formula and Breast Milk.



x : Breast milk = 1.2 ± 0.2 mg/l (2).

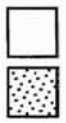
A to P : Each letter represents an infant formula (see Appendix B).

Figure II. The Ratio Between Copper Content in Infant Formula and Breast Milk.



x : Breast milk = 0.25 ± 0.03 mg/l (2).

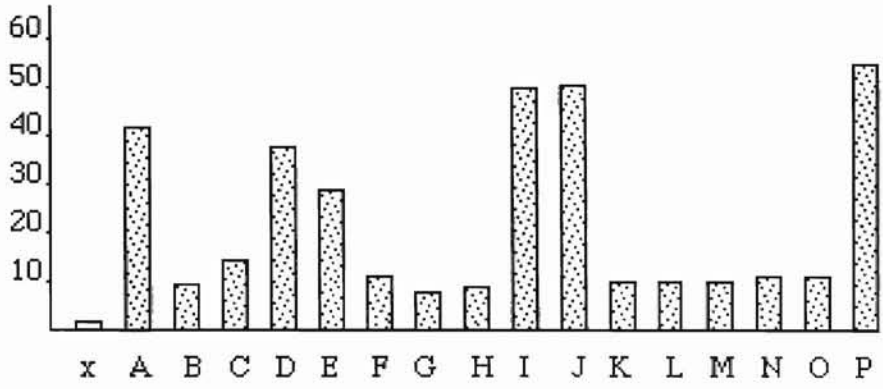
A to P : Each letter represents an infant formula (see Appendix B)



The content in breast milk.

The content in infant formulas.

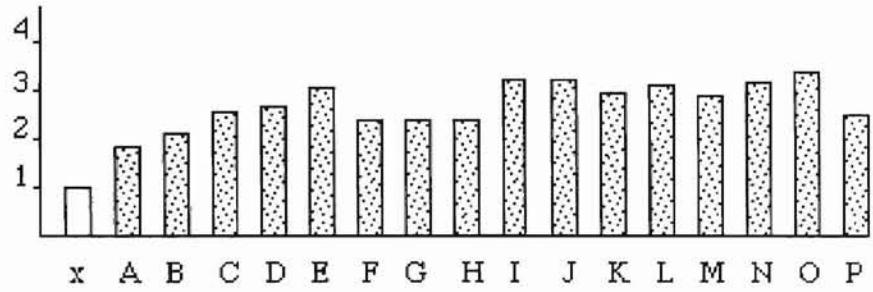
Figure III. The Ratio between Manganese Content in Infant Formula and Breast Milk.



x : Breast milk = 6 ± 2 µg/l (2).

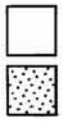
A to P : Each letter represents an infant formula (see Appendix B)

Figure IV. The Ratio between Selenium Content in Infant Formula and Breast Milk.



x : Breast milk = 20 ± 5.0 µg/l (2).

A to P : Each letter represents an infant formula (see Appendix B)



The content in breast milk.

The content in infant formulas.

Table XI. Comparison of the RDA for Zinc for Age Group 0-1 Year Old (5 mg/d) and the Estimated Amount Typically Consumed (mg/d) from Infant Formulas.

Sample	Concentration (±SD), mg/l	Estimated Intake, mg/d (a) (16-32 oz./d)
ALIMENTUM®-Protein Hydrolysate w/Iron (ready)	5.12 (±0.45)	2.42 - 4.83
SIMILAC®PM60/40-Low iron (ready)	5.63 (±0.30)	2.66 - 5.31
SIMILAC® Low Iron (ready)	5.27 (±0.46)	2.49 - 4.97
ISOMIL®-Soy formula w/Iron (ready)	5.26 (±0.32)	2.48 - 4.96
SIMILAC®-Special Care w/Iron (ready)	10.20 (±0.69)	4.82 - 9.65
SIMILAC®-w/Iron (concentrated)	5.28 (±0.21)	2.49 - 4.98
SIMILAC®-Low Iron (concentrated)	5.13 (±0.92)	2.42 - 4.84
SIMILAC®-w/Iron (ready)	5.08 (±0.46)	2.40 - 4.80
ISOMIL®-Soy formula w/Iron (concentrated)	5.23 (±0.39)	2.47 - 4.93
ISOMIL®-Soy formula w/Iron (ready)	5.45 (±0.31)	2.57 - 5.15
SIMILAC®-w/Iron (ready)	5.26 (±0.68)	2.49 - 4.97
SIMILAC®-w/Iron (ready)	5.30 (±0.19)	2.50 - 5.00
SIMILAC®-Low Iron (powder)	5.18 (±0.09)	2.45 - 4.89
SIMILAC®-w/Iron (powder)	5.15 (±0.55)	2.43 - 4.86
SIMILAC®PM60/40-Low Iron (ready)	5.23 (±0.33)	2.47 - 4.93
ISOMIL®-Soy formula w/Iron (powder)	5.05 (±0.28)	2.38 - 4.77

a The average intake in one day in mg.
The average amount of milk consume by infant age 0-1 year old is between 16 - 32 fl.oz.
16 fl. oz. = 16 fl.oz. x 29.5 ml/fl.oz = 472 ml = 0.472 l
32 fl. oz. = 32 fl.oz. x 29.5 ml/fl.oz = 944 ml = 0.944 l
The intake of mineral in mg/d = 0.472 l x amount of mineral (mg/l).
= 0.944 l x amount of mineral (mg/l).

Table XII. Comparison of the ESADDI for Copper for Age Group 0-1 Year Old (0.4-0.6 mg/d) and the Estimated Amount Typically Consumed (mg/d) from Infant Formulas.

Sample	Concentration (±SD), mg/l	Estimated Intake, mg/d (a) (16-32 oz./d)
ALIMENTUM®-Protein Hydrolysate w/Iron (ready)	0.63 (±0.04)	0.29 - 0.59
SIMILAC®PM60/40-Low iron (ready)	0.62 (±0.03)	0.29 - 0.59
SIMILAC®-Low Iron (ready)	0.62 (±0.03)	0.28 - 0.59
ISOMIL®-Soy formula w/Iron (ready)	0.57 (±0.14)	0.27 - 0.54
SIMILAC®-Special Care w/Iron (ready)	1.72 (±0.13)	0.81 - 1.62
SIMILAC®-w/Iron (concentrated)	0.61 (±0.16)	0.29 - 0.57
SIMILAC®-Low Iron (concentrated)	0.63 (±0.08)	0.30 - 0.59
SIMILAC®-w/Iron (ready)	0.61 (±0.04)	0.29 - 0.58
ISOMIL®-Soy formula w/Iron (concentrated)	0.56 (±0.09)	0.26 - 0.53
ISOMIL®-Soy formula w/Iron (ready)	0.58 (±0.03)	0.28 - 0.55
SIMILAC®-w/Iron (ready)	0.62 (±0.11)	0.29 - 0.58
SIMILAC®-w/Iron (ready)	0.60 (±0.04)	0.28 - 0.57
SIMILAC®-Low Iron (powder)	0.66 (±0.05)	0.31 - 0.62
SIMILAC®-w/Iron (powder)	0.63 (±0.03)	0.30 - 0.60
SIMILAC®PM60/40-Low Iron (ready)	0.69 (±0.21)	0.30 - 0.60
ISOMIL®-Soy formula w/Iron (powder)	0.54 (±0.02)	0.26 - 0.51

a The average intake in one day in mg.
The average amount of milk consume by infant age 0-1 year old is between 16 - 32 fl.oz.
16 fl. oz. = 16 fl.oz. x 29.5 ml/fl.oz = 472 ml = 0.472 l
32 fl. oz. = 32 fl.oz. x 29.5 ml/fl.oz = 944 ml = 0.944 l
The intake of mineral in mg/d = 0.472 l x amount of mineral (mg/l).
= 0.944 l x amount of mineral (mg/l).

Table XIII. Comparison of theESADDI for Manganese for Age Group 0-1 Year Old (0.3-1.0 mg/d) and the Estimated Amount Typically Consumed (mg/d) from Infant Formulas.

Sample	Concentration (±SD), mg/l	Estimated Intake, mg/d (a) (16-32 oz./d)
ALIMENTUM®-Protein Hydrolysate w/Iron (ready)	0.25 (±0.010)	0.12-0.23
SIMILAC®PM60/40-Low iron (ready)	0.05 (±0.005)	0.02-0.05
SIMILAC®Low Iron (ready)	0.08 (±0.008)	0.04-0.08
ISOMIL®-Soy formula w/Iron (ready)	0.23 (±0.040)	0.11-0.21
SIMILAC®-Special Care w/Iron (ready)	0.17 (±0.032)	0.08-0.16
SIMILAC®-w/Iron (concentrated)	0.07 (±0.005)	0.03-0.06
SIMILAC®-Low Iron (concentrated)	0.05 (±0.003)	0.03-0.05
SIMILAC®-w/Iron (ready)	0.06 (±0.011)	0.03-0.05
ISOMIL®-Soyformula w/Iron (concentrated)	0.30 (±0.013)	0.14-0.28
ISOMIL®-Soy formula w/Iron (ready)	0.31 (±0.004)	0.14-0.29
SIMILAC®-w/Iron (ready)	0.06 (±0.005)	0.03-0.06
SIMILAC®-w/Iron (ready)	0.06 (±0.006)	0.03-0.06
SIMILAC®-Low Iron (powder)	0.06 (±0.006)	0.03-0.06
SIMILAC®-w/Iron (powder)	0.07 (±0.009)	0.03-0.07
SIMILAC®PM60/40-Low Iron (ready)	0.07 (±0.006)	0.03-0.07
ISOMIL®-Soy formula w/Iron (powder)	0.33 (±0.007)	0.16-0.31

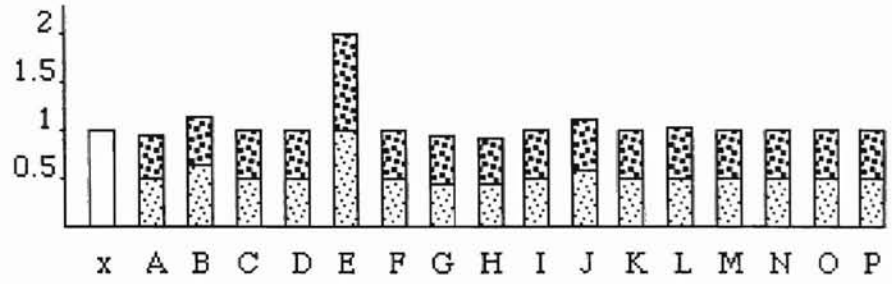
a The average intake in one day in mg.
The average amount of milk consume by infant age 0-1 year old is between 16 - 32 fl.oz.
16 fl. oz. = 16 fl.oz. x 29.5 ml/fl.oz = 472 ml = 0.472 l
32 fl. oz. = 32 fl.oz. x 29.5 ml/fl.oz = 944 ml = 0.944 l
The intake of mineral in mg/d = 0.472 l x amount of mineral (mg/l).
= 0.944 l x amount of mineral (mg/l).

Table XIV. Comparison of the RDA for Selenium for Age Group 0-1 Year Old (10-15 µg/d) and the Estimated Amount Typically Consumed (µg/d) from Infant Formulas.

Sample	Concentration (±SD), µg/l	Estimated Intake, µg/d (a) (16-32 oz./d)
ALIMENTUM®-Protein Hydrolysate w/Iron (ready)	35.33 (±3.40)	16.85 - 33.35
SIMILAC®PM60/40-Low iron (ready)	41.67 (±2.21)	19.88 - 39.34
SIMILAC®-Low Iron (ready)	50.67 (±3.68)	24.17 - 47.83
ISOMIL®-Soy formula w/Iron (ready)	53.83 (±2.12)	25.68 - 50.82
SIMILAC®-Special Care w/Iron (ready)	59.00 (±3.92)	28.14 - 55.70
SIMILAC®- w/Iron (concentrated)	46.33 (±2.36)	22.10 - 43.74
SIMILAC®-Low Iron (concentrated)	45.83 (±2.40)	21.86 - 43.26
SIMILAC®-w/Iron (ready)	48.50 (±2.57)	23.13 - 45.78
ISOMIL®-Soy formula w/Iron (concentrated)	63.50 (±2.25)	30.29 - 59.94
ISOMIL®-Soy formula w/Iron (ready)	63.50 (±2.25)	30.29 - 59.94
SIMILAC®-w/Iron (ready)	55.50 (±3.57)	26.47 - 53.39
SIMILAC®-w/Iron (ready)	62.00 (±4.64)	29.57 - 60.57
SIMILAC®-Low Iron (powder)	55.75 (±2.68)	26.59 - 54.47
SIMILAC®-w/Iron (powder)	64.00 (±1.23)	30.53 - 60.42
SIMILAC®PM60/40-Low Iron (ready)	66.00 (±4.64)	31.48 - 62.30
ISOMIL®-Soy formula w/Iron (powder)	50.00 (±4.12)	23.85 - 47.20

a The average intake in one day in µg.
The average amount of milk consume by infant age 0-1 year old is between 16 - 32 fl.oz.
16 fl. oz. = 16 fl.oz. x 29.5 ml/fl.oz = 472 ml = 0.472 l
32 fl. oz. = 32 fl.oz. x 29.5 ml/fl.oz = 944 ml = 0.944 l
The intake of mineral in µg/d = 0.472 l x amount of mineral (µg/l).
= 0.944 l x amount of mineral (µg/l).

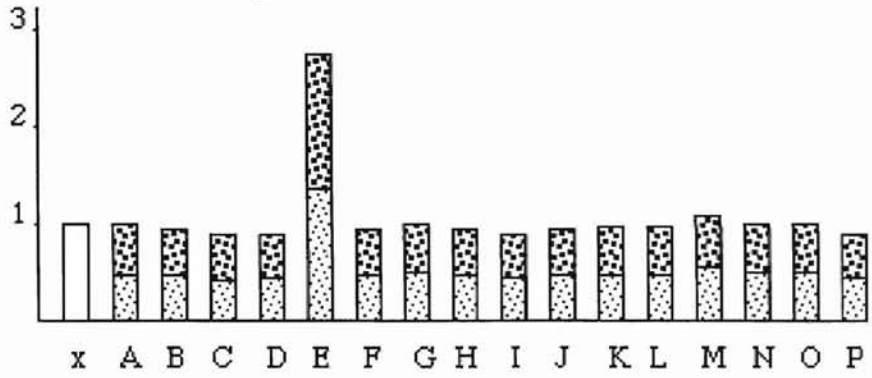
Figure V. The Ratio Between the 1989 RDA for Zinc and the Estimated Amount Consumed (mg/d) from Various Infant Formulas.



x : The 1989 RDA for zinc (5mg/d) for age group 0 - 1 year old.

A to P : A letter assigned to each infant formula (see Appendix B).

Figure VI. The Ratio Between the ESADDI for Copper and the Estimated Amount Consumed (mg/d) from Various Infant Formulas.



x : The ESADDI for copper (0.4-0.6 mg/d) for age group 0 - 1 year old

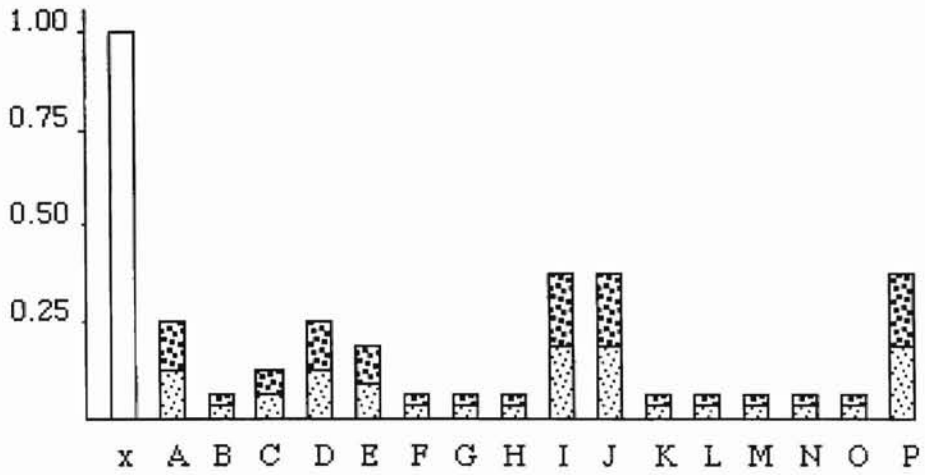
A to P : A letter assigned to each infant formula (see Appendix B).

The intake obtained from a consumption of 32 fl.oz of infant formula in one day.

The intake obtained from a consumption of 16 fl.oz of infant formula in one day.

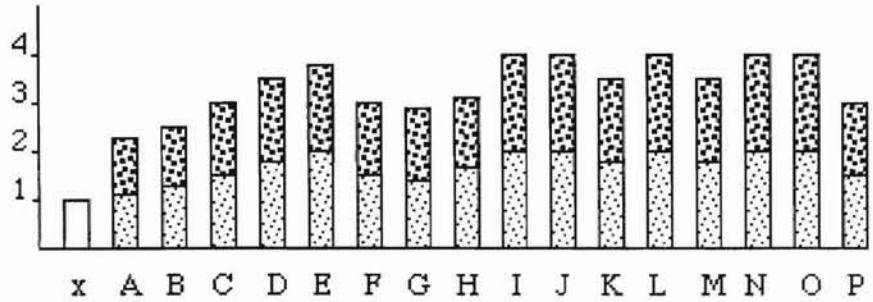
The RDA/ESADDI.

Figure VII. The Ratio Between the ESADDI for Manganese and the Estimated Amount Consumed (mg/d) from Various Infant Formulas.



x : The ESADDI for manganese (0.3-1.0 mg/d) for age group 0 - 1 year old.
A to P : A letter assigned to each infant formula (see Appendix B).

Figure VIII. The Ratio Between the 1989 RDA for Selenium and the Estimated Amount Consumed (µg/d) from Various Infant Formulas.



x : The RDA (1989) for selenium (10-15 µg/d) for age group 0 - 1 year old.
A to P : A letter assigned to each infant formula (see Appendix B).

The intake obtained from a consumption of 32 fl.oz of infant formula in one day.

The intake obtained from a consumption of 16 fl.oz of infant formula in one day.

The RDA/ESADDI.

CHAPTER V

SUMMARY, CONCLUSIONS, AND RECOMMENDATION

Summary

Mineral composition of infant formula is different compared to those in breast milk. The increasing trend towards bottle feeding in the last decade increased the concern towards getting too much or too little of minerals especially those that are not listed in the composition label such as manganese and selenium.

Six different infant formulas in three different forms (ready-to-used, concentrated, and powder) produced by Ross Laboratories (Columbus, Ohio) were used in the experiment. Those were SIMILAC® with iron, SIMILAC®PM 60/40 low iron, ALIMENTUM®Protein Hydrolysate with iron, SIMILAC® low iron, ISOMIL®Soy formula with iron, and SIMILAC®Special care with Iron.

Four different minerals, zinc, copper, manganese, and selenium were tested using the atomic absorption spectrophotometer (Perkin-Elmer 5100 GFAAS - Zeman). Since zinc and copper were measured in mg, the flame side of spectrophotometer was used. Manganese and selenium were found in a smaller amount and were measured in μg. The furnace side of spectrophotometer was used since it is more sensitive and more accurate in measuring mineral present in a very low level.

Three cans or bottles were taken from each lot, and four samples were taken out of one can or bottle to make a total of 160 samples. Each sample went through wet ashing using hydrogen peroxide and nitric acid for one week. All dry samples then were diluted with 1.3 mL of distilled water and 200 μL of concentrated nitric acid. Before analyzing the mineral content, different dilutions for different minerals were made.

The content of zinc, copper, manganese, and selenium in infant formulas being tested were all higher compared to those in breast milk, 4.2 - 8.5 times more for zinc, 2.2 - 6.9 times more for copper, 8.2 - 55.0 times more for manganese, and 1.8 - 3.3

times more for selenium.

The intake of each mineral from infant formulas being tested by average infants with an average consumption of 16 to 32 fl.oz. were also calculated. For zinc, average infants get 0.47 to 1.93 times of the RDA. For copper, average infants get 0.43 to 2.7 times of the ESADDI. For manganese, average infants get 0.02 to 0.31 times of the ESADDI. For selenium, average infants get 1.12 to 4.15 times of the RDA.

Recommendation

Since there were not enough sample available for the experiment, the significant of the results of the experiment cannot be determined. The result reported above should be used to guide further study towards any of these trace minerals to see if they are consistently high in all infant formulas available in the market, and not only in certain lots. There has not been a lot of study being done in mineral absorption in infant formula compared to that in breast milk. For that reason alone, an absorption study to see these differences will contribute more to our understanding about infant formula, to see if it is a good substitute to breast milk.

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APPENDIX

Appendix A. List of Infant Formulas in the Study.

Formula Name	Major Constituent		Iron Content		Form			Container	
	Soy-based	Cow milk-based	Low iron	Added Iron	Ready-to-feed	Concentrated	Powdered	Can	Bottle
SIMILAC® with Iron		x		x	x			x	
		x		x	x				x
	x		x			x		x	
		x		x			x	x	
SIMILAC®PM 60/40 Low iron		x	x		x				x
	x		x				x	x	
	x		x				x		
ALIMENTUM® Protein Hydrolysate with Iron									
SIMILAC® Low Iron		x	x		x			x	
		x	x		x				x
		x	x				x	x	
		x	x			x		x	
ISOMIL® Soy with Iron	x			x	x			x	
	x			x		x		x	
	x			x			x	x	
SIMILAC® Special Care with Iron	x		x		x			x	

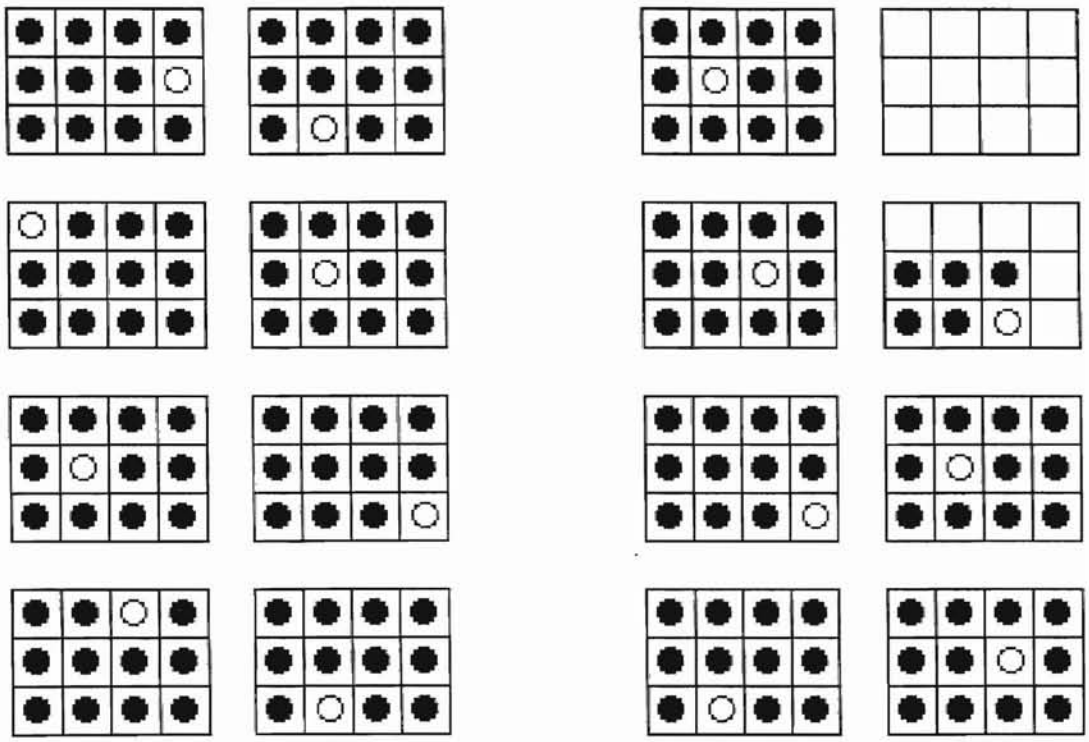
Appendix B. Extra Informations about Infant Formula Used in Study.

Number	Name	Type	Quantity	Vol	Extra Information	Lot #	Exp. date
1 (A)	ALIMENTUM® formula w/ Iron : ready-to-feed	Protein Hydrolysate	6 Quart cans	32 fl.oz	Do not add water	71787RC	Nov. 93
2	SIMILAC®PM60/40 (B)	Low Iron formula : ready-to-feed	48 bottles	4 fl.oz	20 cal/fl.oz Do not add water	71291RN03	Dec. 93
3	SIMILAC® (C)	Low Iron formula : ready-to-feed	4 6-packs	8 fl.oz	Do not add water	65049RD11	Dec. 93
4	ISOMIL® (D)	Soy formula w/ Iron : ready-to-feed	24 cans	8 fl.oz	Do not add water	75826RR00	Apr. 94
5	SIMILAC® (E)	Special Care w/ Iron : ready-to-feed	8 6-packs	4 fl.oz	Add water only if directed by physc.	7505RD01	Apr. 94
6	SIMILAC® (F)	With Iron : concentrated	24 cans	13 fl.oz	Add water	73678RA01	May 94
7	SIMILAC® (G)	Low Iron formula : concentrated	24 cans	13 fl.oz	Add water	73691RE00	May 94
8	SIMILAC® (H)	Low Iron formula : ready-to-feed	6 Quart cans	32 fl.oz	Do not add water	73704RE00	Aug. 94

Appendix B. Extra Informations about Infant Formula Used in the Study (continue).

Number	Name	Type	Quantity	Vol	Extra Information	Lot #	Exp. date
9	ISOMIL® (I)	Soy formula w/ Iron : concentrated	24 cans	13 fl.oz	Add water Milk/Lactose free	73664RE00	Aug. 94
10	ISOMIL® (J)	Soy formula w/ Iron : ready-to-feed	6 Quart cans	32 fl.oz	Do not add water	74665RC	Sept. 94
11	SIMILAC® (K)	With Iron : ready-to-feed	6 Quart cans	32 fl.oz	Do not add water	74772RE00	Sept. 94
12	SIMILAC® (L)	With Iron : ready-to-feed	4 6-packs	8 fl.oz	Do not add water	75087RD01	Oct. 94
13	SIMILAC® (M)	Low Iron formula : powder	6 cans	1 lb.	Add water	71573RB00	Dec. 94
14	SIMILAC® (N)	With Iron : powder	6 cans	1 lb.	Add water	74805RB00	Mar. 95
15	SIMILAC®PM60/40 (O)	Low Iron formula : powder	6 cans	1 lb.	Add water	76266RB01	May 95
16	ISOMIL® (P)	Soy formula w/ Iron : powder	6 cans	14 oz.	Add water Milk/Lactose free	76210RB00	May 95

Appendix C. Tubes Configuration in Heating Block.



The first half

The second half

Tube with sample in it.

Blank tube.

Appendix D. Dry Weight and pH of Samples After Wet Ashing.

Tube #	Sample	Tube wt. g.	Tube+Dry Sample wt. g.	Dry Sample wt. g.	pH
1	A1	5.31379	5.32243	0.00864	6.86
2		5.22038	5.22907	0.00869	
3		5.33630	5.34518	0.00888	
4		5.40381	5.41353	0.00972	
5	A2	5.32799	5.33650	0.00851	6.88
6		5.40953	5.41740	0.00787	
7		5.26929	5.27736	0.00807	
8		5.28210	5.29077	0.00867	
9	A3	5.29491	5.30525	0.01034	6.88
10		5.32711	5.33490	0.00779	
11		5.32778	5.33678	0.00090	
12		5.26381	5.27231	0.00850	
13	B1	5.31016	5.31534	0.00518	6.92
14		5.35320	5.35843	0.00523	
15		5.33268	5.33895	0.00627	
16		5.36602	5.37161	0.00559	
17	B2	5.28993	5.29548	0.00555	7.00
18		5.32433	5.33145	0.00712	
19		5.34467	5.35153	0.00686	
20		5.27524	5.28022	0.00498	
21	B3	5.26252	5.26814	0.00562	6.93
22		5.28706	5.29215	0.00509	
23		5.24714	5.25334	0.00620	
24		5.34670	5.35139	0.00469	
25	C1	5.39852	5.40575	0.00723	6.61
26		5.27308	5.28166	0.00858	
27		5.23609	5.24401	0.00792	
28		5.41907	5.42578	0.00671	
29	C2	5.35927	5.36715	0.00788	6.61
30		5.29572	5.30280	0.00708	
31		5.34429	5.35071	0.00642	
32		5.30950	5.31482	0.00532	
33	C3	5.19662	5.20248	0.00586	6.63
34		5.31583	5.32129	0.00546	
35		5.31901	5.32414	0.00513	
36		5.37587	5.38072	0.00485	

Appendix D. Dry Weight and pH of Samples After Wet Ashing (continue).

Tube #	Sample	Tube wt. g.	Tube+Dry Sample wt. g.	Dry Sample wt. g.	pH
37	D1	5.36814	5.37459	0.00645	6.78
38		5.23711	5.24407	0.00696	
39		5.36128	5.36773	0.00645	
40		5.27698	5.28390	0.00692	
41	D2	5.24777	5.25575	0.00798	6.80
42		5.23021	5.23789	0.00768	
43		5.31377	5.32094	0.00717	
44		5.35660	5.36312	0.00652	
45	D3	5.27788	5.28612	0.00824	6.81
46		5.31878	5.32835	0.00957	
47		5.26316	5.27144	0.00828	
48		5.30187	5.31070	0.00883	
49	E1	5.27751	5.29099	0.01348	6.71
50		5.22297	5.23694	0.01397	
51		5.21985	5.23376	0.01391	
52		5.28304	5.29744	0.01440	
53	E2	5.38495	5.39791	0.01296	6.73
54		5.35388	5.36767	0.01379	
55		5.35161	5.36590	0.01429	
56		5.28503	5.29917	0.01414	
57	E3	5.27836	5.29147	0.01311	6.73
58		5.26202	5.27524	0.01322	
59		5.24704	5.26090	0.01386	
60		5.32760	5.34094	0.01334	
61	F1	5.27556	5.28236	0.00680	6.73
62		5.30028	5.30697	0.00669	
63		5.25729	5.26575	0.00846	
64		5.26196	5.26889	0.00694	
65	F2	5.29913	5.30493	0.00580	6.70
66		5.26356	5.26972	0.00616	
67		5.19173	5.19911	0.00738	
68		5.29454	5.30170	0.00716	
69	F3	5.24424	5.25128	0.00704	6.70
70		5.26724	5.27448	0.00724	
71		5.35439	5.36227	0.00788	
72		5.26631	5.27332	0.00701	

Appendix D. Dry Weight and pH of Samples After Wet Ashing (continue).

Tube #	Sample	Tube wt. g.	Tube+Dry Sample wt. g.	Dry Sample wt. g.	pH
73	G1	5.27125	5.27881	0.00756	6.68
74		5.29236	5.30025	0.00789	
75		5.23209	5.23930	0.00721	
76		5.22818	5.23588	0.00770	
77	G2	5.15495	5.1635	0.00855	6.68
78		5.25484	5.26185	0.00701	
79		5.38393	5.39089	0.00696	
80		5.36974	5.37742	0.00768	
81	G3	5.20513	5.21253	0.00740	6.70
82		5.30212	5.30872	0.00660	
83		5.32377	5.33102	0.00725	
84		5.27969	5.28709	0.00740	
85	H1	5.29869	5.30616	0.00757	6.73
86		5.22835	5.23576	0.00741	
87		5.29236	5.30042	0.00806	
88		5.35749	5.36529	0.00780	
89	H2	5.40292	5.41405	0.01113	6.73
90		5.24941	5.25818	0.00877	
91		5.17700	5.18626	0.00926	
92		5.30529	5.31216	0.00678	
93	H3	5.21725	5.22317	0.00592	6.75
94		5.31242	5.31862	0.00620	
95		5.27883	5.28679	0.00796	
96		5.24170	5.24784	0.00614	
97	I1	5.23680	5.24401	0.00721	6.70
98		5.28907	5.29736	0.00829	
99		5.35280	5.36046	0.00766	
100		5.37713	5.38471	0.00758	
101	I2	5.26470	5.27212	0.00742	6.70
102		5.22515	5.23273	0.00758	
103		5.28948	5.29668	0.00720	
104		5.25378	5.26080	0.00702	
105	J1	5.33783	5.34590	0.00807	6.58
106		5.23594	5.24608	0.01014	
107		5.24154	5.25180	0.01026	
109		5.21084	5.21885	0.00801	

Appendix D. Dry Weight and pH of Samples After Wet Ashing (continue).

Tube #	Sample	Tube wt. g.	Tube+Dry Sample wt. g.	Dry Sample wt. g.	pH
110	J2	5.31294	5.32091	0.00797	6.58
111		5.31242	5.32113	0.00871	
112		5.27121	5.28032	0.00911	
113		5.26800	5.27394	0.00594	
114	K1	5.23951	5.24545	0.00594	6.55
115		5.28482	5.29263	0.00781	
116		5.35282	5.36016	0.00734	
117		5.23736	5.24338	0.00602	
118	K2	5.37774	5.38479	0.00705	6.53
119		5.26811	5.27608	0.00797	
120		5.18499	5.19102	0.00603	
121		5.19141	5.19785	0.00644	
122	L1	5.34438	5.35384	0.00946	6.56
123		5.29994	5.30869	0.00875	
124		5.34788	5.35447	0.00659	
125		5.34695	5.35324	0.00629	
126	L2	5.33971	5.34851	0.00880	6.50
127		5.28465	5.29047	0.00582	
128		5.33094	5.33761	0.00667	
129		5.37604	5.38495	0.00891	
130	M1	5.25632	5.28022	0.02390	6.80
131		5.22217	5.23175	0.00958	
132		5.27047	5.28176	0.01129	
133		5.28047	5.29261	0.01214	
134	M2	5.28809	5.30156	0.01135	6.76
135		5.27886	5.28670	0.00784	
136		5.31650	5.32438	0.00788	
137		5.28745	5.29807	0.01062	
138	N1	5.30793	5.31635	0.00842	6.85
139		5.3374	5.34538	0.00764	
140		5.32882	5.33636	0.00754	
141		5.33576	5.35366	0.00790	
142	N2	5.25948	5.26596	0.00648	6.83
143		5.29457	5.30029	0.00572	
144		5.29035	5.29736	0.00701	
145		5.28880	5.29558	0.00678	

Appendix D. Dry Weight and pH of Samples After Wet Ashing (continue).

Tube #	Sample	Tube wt. g.	Tube+Dry Sample wt. g.	Dry Sample wt. g.	pH
146	O1	5.38141	5.38656	0.00515	6.97
147		5.31889	5.32322	0.00433	
148		5.34713	5.35129	0.00416	
149		5.30876	5.31451	0.00575	
150	O2	5.18965	5.19332	0.00367	6.93
151		5.22572	5.23169	0.00597	
152		5.23820	5.24433	0.00613	
153		5.26303	5.26853	0.00550	
154	P1	5.25227	5.26198	0.00971	6.75
155		5.22399	5.23363	0.00964	
156		5.24804	5.26006	0.01202	
157		5.32180	5.33179	0.00999	
158	P2	5.27348	5.28592	0.01244	6.75
159		5.28407	5.29654	0.01247	
160		5.23301	5.24425	0.01124	
161		5.32948	5.33928	0.00980	
163	B1 *	5.32265	5.32270	0.00005	
164	B2	5.26848	5.26872	0.00024	
165	B3	5.28642	5.28650	0.00008	
166	B4	5.29931	5.29947	0.00016	
167	B5	5.21351	5.21364	0.00013	
168	B6	5.23869	5.23975	0.00106	
169	B7	5.31960	5.32079	0.00119	
170	B8	5.29576	5.29713	0.00137	
171	B9	5.33977	5.33988	0.00011	
172	B10	5.27144	5.27153	0.00009	
173	B11	5.26695	5.36703	0.00008	
174	B12	5.27302	5.27328	0.00026	
175	B13	5.16465	5.16477	0.00012	
176	B14	5.22019	5.22031	0.00012	
177	B15	5.24061	5.24094	0.00033	

Tube 108 and 162 were broke and thrown away.

* Are all the blank tubes.

Appendix E. Atomic Absorption Result Data.

Sample	Tube #	Zn in mg/l	Cu in mg/l	Mn in µg/l	Se in µg/l (a)
A1	1	5.16	0.50	(b)	36
	2	5.21	0.61	241	38
	3	5.10	0.61	239	-
	4	4.84	0.64	246	-
A2	5	5.15	0.64	236	36
	6	5.36	0.66	246	-
	7	5.57	0.64	250	-
	8	5.48	0.65	240	30
A3	9	3.79	0.60	236	-
	10	5.28	0.64	235	32
	11	5.02	0.68	268	-
	12	5.44	0.65	258	40
B1	13	5.08	0.60	48	43
	14	5.76	0.63	47	39
	15	(c)	(c)	(c)	-
	16	5.60	0.61	57	-
B2	17	5.29	0.66	56	-
	18	5.98	0.61	48	41
	19	5.85	0.64	55	39
	20	5.86	0.63	55	-
B3	21	5.91	0.63	54	-
	22	5.15	0.66	(b)	43
	23	5.61	0.58	43	-
	24	5.81	0.57	55	45
C1	25	4.80	0.59	78	57
	26	4.52	0.56	88	-
	27	5.86	0.64	89	51
	28	5.23	0.65	93	-
C2	29	5.27	0.60	81	48
	30	5.59	0.58	80	45
	31	5.62	0.61	(b)	-
	32	4.39	0.59	(b)	-
C3	33	5.39	0.62	96	-
	34	5.81	0.57	80	52
	35	5.53	0.61	90	51
	36	5.21	0.60	92	-

Appendix E. Atomic Absorption Result Data (continue).

Sample	Tube#	Zn in mg/l	Cu in mg/l	Mn in µg/l	Se in µg/l (a)
D1	37	5.24	0.57	278	50
	38	5.21	0.58	271	-
	39	4.89	0.59	223	53
	40	5.53	0.62	213	-
D2	41	5.17	0.60	300	55
	42	5.50	0.64	245	57
	43	5.28	0.62	282	-
	44	5.76	0.62	236	-
D3	45	5.40	0.58	201	54
	46 (d)				
	47	4.50	0.29	265	54
	48	5.32	0.60	196	-
E1	49	10.54	1.74	211	-
	50	10.80	1.81	214	57
	51	10.07	1.80	203	53
	52	10.85	1.55	149	-
E2	53	10.73	1.69	136	-
	54	10.12	1.80	179	-
	55	10.96	1.84	154	58
	56	9.25	1.81	177	58
E3	57	8.66	1.38	124	64
	58	10.69	1.76	144	64
	59	10.21	1.64	140	-
	60	9.52	1.83	209	-
F1	61	5.17	0.38	(b)	46
	62	5.43	0.65	60	44
	63	5.46	0.74	70	-
	64	5.43	0.20	68	-
F2	65	5.38	0.58	70	-
	66	5.38	0.65	74	-
	67	4.76	0.76	56	45
	68	5.19	0.70	61	49
F3	69	5.06	0.49	66	50
	70	5.23	0.68	67	-
	71	(b)	(b)	61	-
	72	(b)	(b)	70	44

Appendix E. Atomic Absorption Result Data (continue).

Sample	Tube#	Zn in mg/l	Cu in mg/l	Mn in µg/l	Se in µg/l (a)
G1	73	2.17	0.62	58	-
	74	5.47	0.70	52	46
	75	5.19	0.65	54	-
	76	5.43	0.64	51	42
G2	77	4.98	0.80	51	-
	78	5.47	0.61	57	47
	79	5.81	0.62	54	49
	80	5.67	0.66	51	-
G3	81	5.30	0.41	50	45
	82	5.16	0.61	48	-
	83	5.38	0.62	53	-
	84	5.53	0.62	48	46
H1	85	3.78	0.56	69	45
	86	5.12	0.66	45	50
	87	5.27	0.54	(b)	-
	88	5.24	0.63	(b)	-
H2	89	5.40	0.62	53	-
	90	5.10	0.56	47	51
	91	5.44	0.61	54	51
	92	5.10	0.63	80	-
H3	93	4.88	0.61	61	-
	94	5.03	0.63	50	49
	95	5.36	0.64	50	-
	96	5.23	0.62	46	45
I1	97	5.19	0.57	266	-
	98	5.29	0.61	301	-
	99	5.45	0.59	298	66
	100	5.36	0.58	304	65
I2	101	5.07	0.63	(b)	63
	102	5.38	0.34	305	60
	103	5.55	0.57	(b)	-
	104	4.50	0.59	296	-
J1	105	5.33	0.54	301	-
	106	5.50	0.61	304	67
	107	5.85	0.59	307	62
	109	5.24	0.59	299	-

Appendix E. Atomic Absorption Result Data (continue).

Sample	Tube#	Zn in mg/l	Cu in mg/l	Mn in µg/l	Se in µg/l (a)
J2	110	4.80	0.56	303	-
	111	5.75	0.63	307	63
	112	5.60	0.64	305	62
	113	5.55	0.60	313	-
K1	114	5.56	0.66	74	-
	115	3.84	0.66	64	51
	116	5.46	0.66	61	59
	117	5.66	0.34	53	-
K2	118	4.42	0.61	56	-
	119	5.64	0.70	66	-
	120	5.69	0.63	61	59
	121	5.84	0.66	66	53
L1	122	5.38	0.56	61	-
	123	5.32	0.66	68	-
	124	5.39	0.64	63	63
	125	5.36	0.62	49	55
L2	126	4.80	0.56	56	68
	127	5.38	0.56	67	-
	128	5.39	0.60	68	-
	129	5.38	0.61	58	62
M1	130	5.13	0.65	(b)	-
	131	5.02	0.63	(b)	-
	132	5.32	0.68	81	54
	133	5.19	0.66	68	53
M2	134	5.20	0.72	46	-
	135	5.30	0.61	55	56
	136	5.18	0.59	58	60
	137	5.13	0.70	55	-
N1	138	5.48	0.61	89	-
	139	5.56	0.59	69	-
	140	5.51	0.67	65	64
	141	5.13	0.70	55	62
N2	142	5.02	0.62	62	65
	143	5.27	0.66	59	-
	144	5.08	0.62	62	-
	145	3.76	0.63	59	65

Appendix E. Atomic Absorption Result Data (continue).

Sample	Tube#	Zn in mg/l	Cu in mg/l	Mn in µg/l	Se in µg/l (a)
O1	146	5.17	0.60	71	62
	147	5.66	0.76	76	-
	148	5.69	0.22	71	-
	149	5.58	0.82	69	61
O2	150	4.98	0.66	62	-
	151	4.82	0.67	63	72
	152	4.98	0.65	62	69
	153	4.95	0.73	78	-
P1	154	5.34	0.54	326	57
	155	4.62	0.54	337	47
	156	4.64	0.54	343	-
	157	5.39	0.58	324	-
P2	158	5.24	0.54	327	47
	159	4.90	0.53	321	49
	160	5.09	0.53	334	-
	161	5.21	0.53	327	-

a. For selenium, 2 out of 4 samples with the closest weight were chosen from each can for mineral analysis.

b. Missing samples.

c. The particular sample was randomly selected for preleminary testing to test the equipment.

d. Sample #46 could not be made into a solution state, therefore was not used for mineral analysis.

VITA

Emilia Elfiranti Achmadi

Candidate for the Degree of

Master of Science

Thesis: SELENIUM, MANGANESE, ZINC AND COPPER CONTENT IN INFANT FORMULA

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