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Determination of Select Metal Ions in Commercially Available Conventional and Organic Baby Foods.

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Determination of Select Metal Ions in Commercially Available Conventional and Organic Baby Foods

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by

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ABSTRACT

Determination of Select Metal Ions in Commercially Available

Conventional and Organic Baby Foods

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The goal of this study is to determine whether or not there is an appreciable difference between concentrations of various metal ions present in conventionally grown and processed and organically grown and processed baby foods. Two prominent, commercially available brands were chosen to undergo comparative studies between both their own conventional and organic varieties of second stage green beans and carrots. Samples were tested for cadmium, calcium, iron, lead, nickel, and zinc. Two containers of each variety of baby food were sampled in triplicate, with purchases of foods being made in separate areas to ensure that each set came from different batches. Samples were digested with nitric acid, appropriately diluted, and analyzed for metal content by flame atomic absorption spectroscopy with the regular standard calibration curve and standard addition method. There was little overall difference in metal content between the conventional and organic foods tested.

DEDICATION

I wish to share the dedication of this work among my husband, Aaron, our daughter, Eilidh Jade, and my late grandfather, Mr. Jack Buchanan.

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I would like to offer my sincere thanks and gratitude to Dr. Ho for allowing me to conduct research under his guidance. He is kind and encouraging and has often kept me from giving up on this endeavor, though he may not be aware of this. He is not an instructor, but a teacher. His inviting demeanor, his care and attention toward his students, and his accessibility will not be forgotten. I appreciate his belief in me, and I hope that I can become a teacher of his caliber in my own career.

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CHAPTER 1

INTRODUCTION

It was not so long ago that all organic products were confined to small corners of select supermarkets, with stores in some areas having no organic offerings at all. This is no longer the case, as now large sections of stores are devoted to wide varieties of organic products, having long since left the confines of the produce section. As organic selections grow, so to do the arguments both for and against them. Perhaps one of the most widely heard arguments is the organically grown and produced products are both healthier and safer than their conventional counterparts [1]. Organic farming is becoming more widely preferred due to the restrictions concerning genetic engineering modifications and the use of growth hormones. However, the consumer is faced with a cost difference between organic and conventional products that may be quite large from time to time. As the world's economy continues to become more challenging, consumers must decide whether the perceived benefits of organic products are worth the added cost. With more and more products offering organic varieties of their long-running conventional counterparts, the question arises as to whether or not these products are better. This also means that there must be some definition of "better". As mentioned above, the growing practices used in conventional and organic farming are very different from one another, and one may also question the techniques involved in processing that take organically grown produce from the fields to a container on a grocer's shelf. Analytically speaking, what do these organic products have (or lack) that conventional products do not?

In seeking an answer to this question, the field was narrowed to an examination of baby food. Ideally, companies producing baby food would be held to such stringent standards that little difference would be seen between their conventional and organic products. However, anything purchased specifically for children is subject to the scrutinizing eye of the parents who want to ensure the best for their children. Having spent those indecisive moments of my own in the supermarket, wondering if I should pay just a little extra for organic, I wanted to see for myself if those extra pennies that add up to extra dollars were justified. To begin moving in that direction, it was first necessary to determine what, exactly, constituted something as "organic" or not.

Organic products are subject to guidelines, regulations, and specifications set forth by the United States Department of Agriculture (USDA). All crops grown organically must employ practices that focus on sustainability through the use practices such as crop rotation, fertilization with animal and plant wastes, and a general emphasis on conservation of water and soil. The use of what some would refer to as growth enhancing techniques, such as genetic engineering, treatment with ionizing radiation, or treatment with sewage sludge, are not allowed. Further guidelines extend to the processing of organically grown produce [2, 3].

Heavy Metals and Human Health

Anything raised in an open environment is, of course, subject to the effects of that environment. Those effects could entail either the deposition of toxic substances or the depletion of nutrients present. For this study, six metals were selected for analysis; three of which are considered toxic heavy metals, and three that are essential metals required by body systems.

Selections were made in this manner in an effort to investigate the claims made concerning organic foods to be better, healthier foods than their conventionally grown counterparts.

Cadmium

Cadmium is a heavy metal that occurs naturally in the earth's crust in mineral form and is found in relatively small quantities in both the atmosphere and the terrestrial environment. Cadmium itself is not mined but is accrued as a by-product of the processing of copper, zinc, lead, and other metal ores, as well as by the burning of fossil fuels [4]. It is used in the manufacture of batteries, as well as in metal-plating and plastics industries [4]. Cadmium is an odorless and tasteless contaminant, and, as such, its presence is only detectable via chemical analysis.

Cadmium enters biological systems in the characteristic manner associated with other heavy metals, such as mercury or lead [5]. As a component of industrial emissions and fossil fuel exhausts, the element is spewed into the atmosphere where it is spread across larger areas via wind currents. Particulates generally settle into the soil or surface water, where they become absorbed by both terrestrial and aquatic plants and animals. As with mercury, aquatic life is particularly susceptible to cadmium uptake, which warranted fishing advisories and restrictions by the United States Environmental Protection Agency (EPA) for several coastal areas in the northeastern United States [5]. Additionally, cadmium may also accumulate in crops, particularly leafy vegetables, through roots of plants upon deposition [6]. Metabolic processes of organisms cannot efficiently rid systems of cadmium, making it difficult to decrease levels within tissues [7]. Humans subjected to cadmium poisoning must seek medical treatment in order to efficiently remove the metal, usually through some form of chelation therapy.

The primary sources of cadmium exposure are eating contaminated foods or smoking [4]. Introduction of the metal into the body occurs most commonly from eating contaminated grains or vegetables, particularly those grown where phosphate fertilizers or sewage sludge was applied to fields [4]. The most common manner in which inhalation of cadmium occurs is through smoking or exposure to second-hand smoke. Cadmium is known to accumulate in tobacco leaves during the growing process, and studies published by the Division of Toxicology and Environmental Medicine have shown smoking to consistently double the levels of cadmium in the blood [6]. Smoking a single pack of cigarettes per day can allow for the absorption of between one and three micrograms of cadmium [6]. Though second-hand smoke exposure would most likely be associated with lower cadmium exposure, children exposed to second-hand smoke on a regular basis would most likely be susceptible to its effects. Though less common, cadmium may also enter the body through the respiratory system from airborne sources, such as industrial emissions [6].

Cadmium inhalation may lead to respiratory distress and severe lung damage. Ingestion of high levels of cadmium may lead to stomach lining irritation, thereby inducing vomiting and diarrhea [4]. The most severe effects of exposure generally occur in the long-term and may be seen in various body systems. Long-term exposure damages both the liver and kidneys, as the key functions of these organs are in removing toxins from the body. Individuals have the potential to develop kidney disease or suffer from kidney failure due to the damaging of the proximal tubules of nephrons within the kidney, which disrupts the filtration process and allows proteins and essential ions to leak into urine [4]. In turn, various deficiencies, such as low calcium levels leading to fragile bones and possible osteoporosis, may also be observed [8. 9].

Cadmium may also damage olfactory senses, lead to the discoloration of the teeth, or cause anemia [6].

Lead

A stable product of the decay of both thorium and uranium, lead is the most abundant heavy metal found in the earth's crust and is highly toxic to body systems [10]. Like other heavy metals, lead occurs naturally throughout the environment, but the levels are enhanced by mining, industry, and the burning of fossil fuels. Though its uses have come under scrutiny in the past several decades, lead is applicable in a wide range of processes and materials. Used in the production of various metal products, of leaded fuels, of batteries, etc, the wide variety of applications has resulted in the prevalence of lead as an environmental pollutant and human health toxin. It is the introduction of lead by human activities that has thrown the natural lead cycle off balance, allowing for atmospheric levels that are much higher today than even a century ago [10]. Larger particles settle to the ground, polluting surface water and soils. Smaller particles remain in the atmosphere for varying lengths of time, eventually falling to the earth with precipitation [10].

When working directly with lead in any industrial process, the risk of exposure for that particular individual is greatly increased. However, it is the exposure of the population at large that is of concern here. The use of lead in household pipes and paints has been restricted for several decades now, therefore, accounting for less of the newer exposure cases. As with most heavy metal toxins, the risk of exposure is highest in terms of dietary vectors. Lead that is deposited in the soil is taken up by plants, where it then resides due to the inability of the plant to process or metabolize the metal. Though it is a toxin, it has been shown that plants can

accumulate quantities of lead that far exceed levels permissible in humans [3]. The human digestive system is unable to metabolize lead; therefore, levels of lead taken in through the diet accumulate over time. It is a combination of the environmental permeation coupled with the ability of lead to bioaccumulate up trophic levels of the food chain, i.e. from primary to higher level consumers, that makes it particularly dangerous.

Lead is a known neurotoxin and has been shown to wreak havoc on human body systems [10]. Exposure disrupts normal cell function, with effects covering a wide range depending upon exposure levels. Allowable levels in adults and children vary greatly, as adults seem to be able to better manage exposure. The effects of lead tend to be magnified in infants and children, as their systems are still in developmental stages and have been linked to problems ranging from stunted cognitive development and diminished IQ to metabolic issues to system failure or death. Consequently, the United States Center for Disease Control (CDC) lowered the allowable amount of blood lead levels in children six months to five years of age from 25 µg of lead /dL to only 10 µg of lead /dL [11]. The effects of lead on the developing fetus are of concern and have been the focus of recent studies due to the storage of lead in skeletal tissue of women of child-bearing age [10, 11, 12].

Nickel

Nickel is generally considered a trace element and is found in soil, in meteorites, and in volcanic emissions. Most commonly nickel is found in the earth as either an oxide or sulfide compound. Nickel's environmental presence is exacerbated by industrial process, by oil and coal-burning facilities, and by waste incineration processes [13]. Once deposited, nickel has a high affinity toward iron and manganese and tends to strongly attach to ores containing those

metals. Generally a soil contaminant, nickel has not been shown to accumulate in the tissues of animals that would be consumed by humans [13].

As is the case with other heavy metals, nickel exposure tends to occur most often through dietary means. Reactions to nickel have been seen at varying levels, and it may illicit allergy-type symptoms, such as itching or burning at the contact site. Reaction symptoms may increase in severity with continued exposure. Though rare, reactions to nickel may manifest as asthmatic attacks. In general, there is no means of curing nickel sensitivity other than avoiding contact with the metal [14].

The reactions mentioned above generally occur with skin contact to nickel or nickel plated objects. Nickel ingestion produces effects similar to those associated with cadmium or lead and must be treated through chelation therapy [13].

Iron

Iron is an abundant element, exceeded only by aluminum, silicon, and oxygen. An essential metal, iron is an integral component of many proteins and enzymes, such as hemoglobin, that keep biological systems functioning properly and is essential to normal cell growth and differentiation [12].

Iron deficiencies are generally more common than overloads. Iron is the core of the metalloprotein hemoglobin, which is involved in oxygen uptake and transport. Low levels of iron interfere with the oxygenation of the body, resulting in fatigue and immune system suppression. In infants and children, iron deficiencies can be more problematic as normal growth and development would be disrupted [12].

The recommended daily allowance of iron for adults is 18 mg, with the American Academy of Pediatrics recommending 11 mg per day for babies and young children [12]. Most dietary iron comes from meat, as the human body is better able to absorb iron from animal sources than from plant sources. Babies and young children generally obtain iron from vegetables, such as green beans, that are often fortified with iron in order to ensure sufficient uptake.

Calcium

An Alkaline Earth Metal, calcium is a rather reactive metal that is present naturally in the earth's crust as compounds instead of its uncombined form. In terms of health, calcium is essential in numerous bodily functions including bone growth and health, blood clotting, blood pressure regulation, and as a part of the ion balance that deals with muscle and nerve control.

Healthy diets should include various sources of calcium for both adults and children. Adults require calcium in order to prevent bone loss, though bone mass is no longer built past the late twenties or early thirties. Calcium deficiencies among children greatly impact growth. It is therefore of utmost importance that the diets of babies and small children be infused with foods rich in calcium in order to ensure healthy bone development [12].

Zinc

Zinc is one of several essential elements necessary to humans in trace amounts. Like many metals, zinc occurs naturally in the environment, but its presence is exacerbated by humans. Large amounts of zinc are released into the environment through such activities as mining, the burning of fossil fuels, the refining of ores, and steel production. Zinc deposition

occurs in the same fashion as other heavier metals in that it is released into the atmosphere and settles into soils and surface waters or remains trapped in the atmosphere before falling to the ground with precipitation. Unlike the other elements studied here, zinc is more likely to build up in animal tissue rather than be taken up by plants [15].

As an essential element, zinc is present in the body in various places and forms. Zinc can be found as a component of skeletal muscle, of bones, of the choroid of the eye, and of prostatic fluids [16]. Zinc is also an integral part of various enzymes and metalloproteins that are involved in both the synthesis and decomposition of the major classes of biomolecules (carbohydrates, lipids, proteins, and nucleic acids) and in metabolic processes associated with many nutrients. Zinc is of great importance as it is involved in the nucleotide transcription processes, is an immune system booster, and contributes to the overall maintenance of cells and organ systems [17].

CHAPTER 2

RESEARCH ON METAL ACCUMULATION ASSOCIATED WITH FARMING

Conventional Farming Techniques

Conventional farming techniques have remained largely unchanged for many years, with most deviations being those that increased crop yields. As the global populations continues on its path of exponential growth, the prevailing attitude in the vast majority of industry seems to center around "more". There are more and more mouths to feed, which calls for more and more food to be made available, which leads to more and more crops that must be cultivated, treated with pesticides, and managed by machinery. When the overarching goal is mass production, the path of least resistance is to use whatever means that make the goal attainable.

Conventional farming supplies consumers both directly and indirectly by selling to both supermarket chains and food processing companies. To meet these demands, large farms are manned with all manner of equipment and machinery to increase the efficiency of managing such vast tracts. Tractors and various attachments plow, sow, fertilize, spread pesticides and insecticides, and harvest the crops. The shift in farming techniques in the U.S. becomes most evident following World War II, and has increased dramatically in the last 65 years. In 1995, the U.S. used a staggering 45 million tons of chemical fertilizers and 770 million pounds of synthetic pesticides [18, 19]. Approximately 95% of all crops produced in the United States today are grown using chemical treatments of sorts, thus holding as the conventional norm [20].

In such methods, crops are exposed to large quantities of chemicals that are not only themselves harmful to human health but that may contain trace amounts of heavy metals due to

manufacturing techniques. A further concern is the deposition of heavy metal particulates from the burning of fossil fuels that power the equipment used. Crop rotation is generally not employed in conventional means, with naturally occurring nutrients depleted after several successive seasons. The lack of nutrients changes the texture and behavior of the soil, making plants grown there more susceptible to the uptake of harmful chemical compounds and heavy metals. Farm and field location is of importance as well, with large fields often being in close proximity to high traffic areas, such as interstate highways.

Conventional methods are effective at mass production. However, the long-term effects of this methodology are only now becoming a concern. Plants themselves are efficient chemical receptors, and the chemicals used to help them grow and protect them from pests often are taken up by the leaves, stems, roots, or fruits. Once taken up by plants, deposition of chemicals becomes virtually permanent, thus offering a vector for chemical contaminants and heavy metals to be introduced to consumers.

Organic Farming Techniques and Regulations

In order to define organic farming techniques, it is necessary to define what the term "organic" really means. In October of 2002, the United States Department of Agriculture (USDA) provided a definition for organic food, stating that organic foods are those produced by farmers who seek to focus on and emphasize the use of renewable resources and soil and water conservation in an effort to enhance the quality of the environment for future generations [20]. Organic production practices seek to maintain biodiversity and ecological balance, sustainability, natural fertilization and pest management practices, and soil integrity and fertility [2, 21].

The USDA has set forth policies governing the organic farming industry that outline crop standards and production practices involving any product that is labeled and marketed as being organic [2]. Stringent regulations have been set in place by the U.S. National Organic Program, a division of the U.S. Department of Agriculture. In terms of crop standards, the guidelines in place are markedly different from the techniques employed by farms that adhere to the conventional methodology of farming. Inspections are first carried out to ensure the integrity of the land being used and to verify that no prohibited substances were used on the field at least three years prior to harvest. Further guidelines govern cultivation and fertilization methods to maintain the fertility of the soil and the crop nutrients [21]. For instance, organic farms must employ techniques such as crop rotation, the usage of cover crops to prevent soil erosion, and the use of animal and plant waste materials as fertilizers. Organic farming operations are limited in their use of synthetic materials, with a list of allowed materials being published and regulated by the USDA [22]. Furthermore, the use of most chemical farming agents, genetic engineering techniques, ionizing radiation, or sewage sludge is not allowed [21].

Organic regulations are not limited to the farm itself but extend to the processing side of industry. Handling standards were also set forth by the USDA to ensure that the practices employed in the growing process were not negated by unsatisfactory processing procedures [21]. For instance, all substances used in processing and production must be approved by the National Organic Program [19, 21]. Organic and non-organic products must not be allowed to mix during production, thus requiring companies to either implement stringent quality control measures between batches or to dedicate certain facilities solely to the processing and production of organic products.

The USDA extends its regulations to the labeling of products, thus addressing the issue of misleading or misrepresenting the contents of a package [19]. Labeling standards are based on the percentage of organic ingredients in the product. All products termed as "organic" must contain from 95% to 100% organic ingredients, and will have the USDA organic seal on the packaging.

All products termed as being "made with organic ingredients" must contain at least 70% organic ingredients and must list up to three of those organic ingredients on the principal or primary label. As these products are not fully organic, the USDA organic seal does not appear on the packaging [2, 21]. All companies seeking an organic designation must be approved by the USDA. Certification standards lay out requirements that must be met by organic production and handling operations. All certification standards must be met in order to receive organic accreditation. Accreditation standards are also in place for those wishing to become USDA-accredited certifying agents [2, 21].

Of additional note in this study was the internal standards in place in the plants of the companies whose products were studied. Both companies employed protocols above and beyond government standards, with protocols being applied to the processing of both conventional and organic baby foods. The conventional and organic varieties produced by each company were processed in either separate facilities or separate lines within a larger compound facility. The company designated as "Brand G" in this study has an agricultural program under the umbrella of the larger corporation that requires outside growers to be full partners and follow all additional practices and procedures outlined by the corporation. A strict and seemingly stream-lined quality control practice is also in place to trace products back the farm from which they originated. The company designated as "Brand B" is family owned and operated with a

portion of their produce being grown within the company itself, thereby requiring fewer outside suppliers. "Brand B" publicizes its certification by the International Organization for Standardization (ISO), as well as its being the first manufacturer of baby foods to operate plants certified in terms of the Leadership in Energy and Environmental Design (LEED). LEED is a nationally accepted, third-party program that certifies green techniques employed by industries [23].

Sample Treatment Techniques

Various instrumental methods can be employed to determine trace concentrations of metals present in samples. The initial challenge in using any spectroscopic method lies in finding sample treatment techniques that transform the sample into a suitable form for analysis. When preparing samples it is important to ensure the dissolution of the sample matrix so as to release metals into solution for analysis. It is also important to either dilute or concentrate the metals present to bring them into a suitable concentration range that coincides with the detection limits of the instrument being used, and to separate the specie or species in question from substances that could pose an interference [24]. The goal of sample preparation is, most often, to produce a homogeneous solution so as to avoid the problematic effects of particulates within a solution. For this reason, a combination of acid digestion and sample ashing are common practice in sample preparation, offering what may be deemed a path of least resistance from undissolved sample to a homogeneous solution ready for analysis.

Dry Sample Ashing

Dry sample ashing, employed predominately for the treatment of organic matter samples to be analyzed for nonvolatile metals, is often used when analyzing for dietary nutritional elements such as calcium, iron, magnesium, manganese, or potassium [24]. Samples are placed in a crucible made of any variety of heat-resistant materials such as pyrex glass, porcelain, silica, or platinum and ignited in a muffle furnace. Organic matter is burned away, leaving metals behind due to their much higher volatilization points.

Dry ashing removes organic matter from a sample quite effectively and is generally much faster than wet digestion methods. However, it is also likely that elements of interest could be lost due to volatilization or that samples could become contaminated by air borne particulates as it is necessary to leave samples open to the atmosphere [24]. Though there is no simple fix for the contamination aspect, it is possible to minimize volatilization by using minimum temperature settings for ashing of samples.

Wet Sample Digestion

Wet digestion of samples is the simplest and most effective digestion method employed widely for sample treatment and preparation [24]. Samples may be digested in an open container, a sealed and pressurized container, or possibly in a microwave. In general, samples are digested in a strong acid that will thoroughly disrupt the normal form so as to completely dissolve all particles, thus releasing elements into solution that would normally be bound within some sort of matrix. In the case of an organic matrix, an oxidizing mixture is used to destroy the carbon-based matrix, giving rise to a clear solution containing metals of interest [24]. Inorganic

samples such as soil samples, rock samples, sediment mixtures, etc. may be digested in varying mixtures of dilute or concentrated acids, depending upon the components of the sample [24].

Digestion of samples by microwave has several advantages over the digestion of samples in open containers. Contamination of samples by air borne particulates is greatly reduced due to the sealed containers specifically designed for this digestion method. These containers also reduce the possibility of the loss of elements due to evaporation or volatilization, as the entire system is closed. Electronic controls and settings on modern microwave digesters allow for conditions that are easily reproducible [24].

CHAPTER 3

ANALYTICAL TECHNIQUES

As the focus of this study centered on the analysis of samples to determine the concentration of various metal ions at presumably trace levels, choosing appropriate analytical instrumentation was of the utmost importance. In terms of modern analytical chemistry, a host of instrumental tools are now available, each with its own characteristics that serve to suit it for a particular type of analysis. The selection of a means of instrumental analysis depends upon a host of factors, such as the type of samples being analyzed, the methods of sample preparation employed, and the information being sought through analysis [25].

Colorimetry

Though modern colorimetry has evolved with technology, the method itself is perhaps one of the oldest analytical techniques employed by chemists as it uses color changes occurring due to absorption of light in the visible region. Colorimetry experiments are known to have been carried out with natural light as the source. The color produced in the sample in question was then visually compared with the coloration seen in a control whose concentration was known [26]. Though subject to the interpretation of the observer, this technique did prove useful in measuring the concentrations of substances present in solution in terms of light absorbed and transmitted by the sample.

The basis of colorimetric analysis associates coloration with concentration. The primary goal of colorimetry is determining the concentration of an unknown by comparing the absorption

and transmission of light by a sample and judging the results against those seen with a solution of known concentration [27]. In visual colorimetry, the source employed is generally white light, natural or artificial, and results are determined using a color comparator. Visual colorimetry is a less satisfactory method of analysis, as determinations may be affected by the color interpretation of the individual making the observation [27]. More valid results may be obtained with a photoelectric colorimeter in which a photoelectric cell takes the place of the human eye. In a photoelectric colorimeter, the light source is contained within a small range of wavelengths, thus subjecting the sample to a limited spectral region [27]. In this set-up, scans may be performed to determine the wavelength at which the most light is absorbed. Once the wavelength of maximum absorbance is known, the concentration may be determined [25].

Colorimetry uses the visible region of the electromagnetic spectrum to measure the response of an element or compound when exposed to certain wavelengths of visible light. Due to the limitations of the visible region, this method is only applicable for elements that absorb radiation within the correct range of wavelengths, thus narrowing the field of substances for which this method of analysis could be employed. However, where colorimetry could be employed, it is a fast method of analysis that does not damage the sample and can be used to accurately determine concentrations [28].

Neutron Activation Analysis (NAA)

Neutron Activation Analysis (NAA) is a technique that may be used to both qualitatively and quantitatively analyze elements present in samples. Though the sensitivity of this method varies from one element to the next (detection limits range from 1 ppb to 1000 ppm), NAA is

capable of analyzing multiple elements simultaneously, with the ability to identify approximately 60 elements [26].

Analysis by neutron activation is accomplished by the irradiation of a sample with neutrons and then measuring the resultant radioactivity [25]. Incident neutrons are captured by target nuclei to form compound nuclei that may release prompt gamma rays upon de-excitation. The radioactive nuclei generated decay, releasing beta particles and delayed gamma rays [25]. Though measurement of prompt gamma rays is sometimes employed, measurement of the delayed gamma rays is the more common practice [25].

Neutron activation techniques generally employ one of three sources; reactors, radionuclides, or accelerators [25]. Radionuclides are considered the most convenient sources for NAA and are relatively inexpensive when compared to other sources. However, reactors producing neutrons via fission of uranium provide the highest available sensitivities for most elements [26].

This particular technique requires the chemist to work around gamma radiation and is therefore draped with various precautionary protocols. Analysis of samples in this manner requires a neutron source - such as reactors, accelerators, or emitters - a gamma ray detector, and a thorough knowledge of interactions and reactions that occur between neutrons and target nuclei. Due to the nature of bombarding samples with neutrons, solid samples are preferred for this type of analysis [24].

X-Ray Fluorescence (XRF)

A spectral property of some substances, X-ray fluorescence (XRF), is widely accepted as an accurate means of providing qualitative and quantitative information concerning sample

composition [26]. In terms of XRF operation, samples are irradiated with an X-ray beam followed by observation of the resulting fluorescence given off by the sample [26]. Samples may be analyzed quickly, require minimal preparation, and are not destroyed by the analytical process [25].

X-ray fluorescence has a wide applicability in the analysis of metals, glass, and ceramics, particularly in industrial productions, and may be used to analyze solid or liquid samples [29]. Material analyzed by X-ray fluorescence are exposed to either X-rays or gamma rays, which causes an electron to be ejected from an atom's inner orbitals. When an electron is ejected in this manner, a hole is created, causing the atom to become unstable. An electron from an outer orbital falls to fill the hole, which results in energy being emitted from the atom in the form of a photon. The released energy is equal to the energy difference between the orbitals and is specific to the element. Thus by measuring the energy released, sample composition can be determined [29].

Anodic Stripping Voltammetry (ASV)

Methods of voltammetry apply a varying potential difference between an indicator electrode and a reference electrode. Oxidation or reduction reactions are induced on the surface of the indicator electrode, with the electrical current increasing sharply when the analyte undergoes either reduction or oxidation [26].

The process of analysis by ASV consists of two steps. In the first step, known as preconcentration, the analyte solution is electrolyzed such that ions of metals are reduced to form an amalgam with the mercury on the electrode. Secondly, the electrical potential is then increased in order to strip the metal ions from the electrode such that they are reoxidized back

into solution, a process referred to as redissolving [26]. As a result of the preconcentration step, ASV is an electrochemical analysis technique with the lowest detection limits for trace metals, ranging from parts per billion (ppb) and possibly lower [25, 29]. It is entirely possible to carry out analyses that yield results in the 10-6 to 10-9 M range [25]. ASV is an attractive technique due to its relative simplicity, its sensitivity, and its rapidity of analysis. However, ASV yields the most highly accurate results when all reagents used are ultrapure and measurements are carried out methodically [29].

Atomic Emission Spectroscopy (AES)

Atomic emission spectroscopy (AES) is a type of spectroscopy that may be used to determine analyte concentrations by measuring energy emissions. Mechanically, the instrumentation set-up is similar to those found in atomic absorption spectrophotometers. Samples are aspirated into an atomization source that provides enough energy to excite atoms in the ground state to higher energy levels. Excited state atoms decay to lower energy states or the ground state by emitting radiant energy. Emission wavelengths can then be analyzed to determine both qualitative and quantitative information about the sample. The principle difference, and major advantage, of AES lies in the ability to analyze for multiple elements at the same time, meaning that Specific hollow cathode sources are not necessary. Because all atoms in the sample are excited simultaneously, their behavior becomes synchronized, allowing for simultaneous detection using a polychromator and multiple detectors [29].

The emission spectrum of an element is a bright line spectrum, with specific lines visible at specific emission wavelengths. Emission spectra can be used in the qualitative identification of elements, as no two elements share the exact same emission patterns. Emission wavelengths

may also be used to determine concentrations of elements present in a sample by measuring the intensity of light emitted at the characteristic wavelength of the element in question [27].

Emission spectroscopy offers several advantages that absorption methods cannot. For instance, emission techniques require higher temperatures, thereby lessening the impacts of chemical interferences or matrix effects [25]. Emission spectroscopy also allows for the simultaneous analysis of elements, including some nonmetals, as satisfactory emission spectra can be generated for many elements under the same conditions. Atomic absorption techniques require specific lamps to be used for each element in question, causing analysis to be done one element at a time [25].

Though advantageous in many respects, it is unlikely that emission methods of analysis would completely replace atomic absorption methods. As mentioned above, chemical interferences and matrix effects are of minimal concern in emission techniques. However, background emission is problematic and requires careful correction [25]. In addition to the skill required in order to produce satisfactory analyses, atomic emission equipment is quite costly in terms of both initial set-up and general operation.

Graphite Furnace Atomic Absorption Spectrophotometry (GFAAS)

In GFAAS, a graphite coated furnace is used in place of a flame to atomize samples. This method is comparable to Flame AAS in that it is simple, quick, and may be used for a large number of metal analytes. Samples are deposited into a graphite tube where they are heated to vaporization. Atomization happens more slowly with GFAAS than with flame methods, as heating occurs in stages. This allows for vapors coming from the sample matrix to be carried away, thus reducing matrix effects and generating signals with greater levels of reproducibility

[25]. Smaller sample sizes are required for this method, and it has detection limits that may be 10 to 100 times beyond those of the flame AAS [30].

Flame Atomic Absorption Spectrophotometry (AAS)

A highly effective means of instrumental analysis, the flame AAS methodology as it is known today, was developed by a team of scientists in the 1950s [29]. A liquid sample is atomized using a flame and is then analyzed for metallic species present. Flame AAS is a sensitive technique that can determine concentrations of metallic species in parts per million (ppm) in both inorganic and organic samples. Should lower detection limits be required, the flame could be replaced with a graphite furnace as the source of atomization [29].

Flame AA instruments must have a specific light source that produces enough energy to excite the sample, a flame cell for sample atomization, an aspirator to introduce the sample to the flame, a monochromator, a detection system, and a readout [29]. Most AA instruments use hollow cathode lamps as radiation sources, which consist of an anode, a hollow cathode constructed of the metal being analyzed, and a quartz or pyrex window through which the light generated can pass. The lamp itself is sealed with either neon or argon inside at a pressure between 1 and 5 torr. Hollow cathode lamps are classified as line sources, thus requiring that each element under analysis have a specific lamp [29]. Although there are a limited number of elements that can be analyzed for using a multi-element lamp whose cathode is constructed of similar elements, the single-atom lamps tend to perform better and produce spectra with less interferences.

Most AA instruments tend to use air-acetylene flames to atomize samples, with acetylene being the fuel and air being the oxidant [25]. This type of flame has the ability to dissociate most

elements for which AAS is applicable, with a flame temperature of approximately 2500 K [31]. It is important that the mix of fuel and oxidant be able to generate a flame that is sufficient for atomization. Equally important is the manner in which samples are introduced to the flame is efficient and reproducible. Samples should be introduced without interference to ensure accurate readings [26].

Atomic absorption spectra are termed as continuous bright line spectra and are generated based on a measure of the amount of light passing through a sample. In order for this spectra to be viewed and for absorption to be quantified, the analyte is introduced into a flame where it is vaporized, dissociating, completely or partially, into the gaseous forms of the constituent elements. A source of specific wavelength is used to excite atoms in the sample from the ground state to an excited state of higher energy. The intensity of the transition generated depends on the concentration of the ground state atoms present. By controlling the lamp and wavelength used, it is possible to analyze samples for trace amounts of various elements.

Absorption and concentration are related via the Beer-Lambert Law, $A = \varepsilon_0 bc$. In the given equation unitless absorbance is represented by A, ε_0 is the molar absorptivity, and b is the path length in cm. According to this relationship, absorbance is directly proportional to concentration [32]. As such, a calibration curve may be generated by measuring the absorbance of standard solutions of known concentrations, and then plotting these measures versus concentration. A linear relationship should result, and the resulting equation can be used to determine analyte concentrations in the unknowns being studied. Modern instruments have the ability to generate calibration curves and calculate concentrations automatically.

In terms of the study conducted here, flame AAS was chosen based on its ability to successfully analyze samples for the metal ions of cadmium, lead, nickel, calcium, iron, and zinc.

The detection limits associated with the elements in question are 0.001 ppm for Cd, 0.008 ppm for Pb, 0.003 ppm for Ni, 0.001 ppm for Ca, 0.006 ppm for Fe, and 0.001 ppm for Zn [25]. These detection limits are more than sufficient with regards to either recommended values for nutrient metals or concern levels for toxic metals. Furthermore, in medical cases where heavy metal exposure is of concern, urine samples are analyzed by flame AAS, with the results helping to determine levels of exposure [33].

Quantitative Analysis Method

The purpose of this study was to quantify levels of various metal analytes in samples obtained from conventional and organic baby foods. Chemical concentrations must be determined by indirect modes of measurements, involving both directly measuring absorbance and creating calibration curves. The concentration of an unknown is the determined by using regression equations from the calibration curves generated.

External Standard Method (with Calibration Curve)

The external standard method involves the preparation of standard solutions of known concentrations from stock solutions. Standards may then be run on an atomic absorption spectrometer to obtain an absorbance measure. Plotting absorbance versus concentration yields a linear relationship that can be used to determine the concentration of analyte in the unknowns [29]. As calibration curves are the basis for determining concentrations in the unknowns, a high degree of linearity is extremely important.

The calibration curves generated for the metals in question are provided in Appendix A and include linear regression equations and correlation coefficient values.

Standard Addition Method

The calibration curve, or external standard, method for analysis by AAS may not always provide an accurate concentration of the unknown. The standard addition method is used to alleviate the problems that may arise. When performing a standard addition study, known volumes of a standard solution are added to aliquots of the unknown. Experimentally, two different volumes of known concentration of the analyte standard solution should be added to aliquots of the unknown. An aliquot is also prepared that contains only the sample in question, with no standards being added in order to create a baseline. All aliquots were then analyzed via AAS, such that a relationship between absorbance and concentration could be established.

Objectives of Research

Regular or organic? Consumers who are concerned with health and wellness should always buy organic, correct? These questions arise with each visit to the supermarket. According to many, organically grown and processed products are deemed far better than their conventional counterparts. Conventionally grown and processed products are presented as being more likely to contain potentially harmful substances due to the chemical-laden techniques by which they are brought to grocery store shelves. Though the techniques employed by organic farmers and processors are different than the conventional norm, there is still the question as to whether or not this is enough to produce significant differences in levels of harmful substances that many believe permeate the atmosphere, water, and soil beyond removal.

It was with the above questions in mind that the subject of this study was formulated. Products for infants and children are subject to what some would perceive as rather devious marketing approaches. With the debate between conventional and organic being a hot topic,

parents, and their wallets, are faced with a dilemma when purchasing beginner vegetables and fruits for their children. In 2007, CNN released its "Planet in Peril" documentary concerning population and environmental issues. In the documentary, it was presented that in body burden studies of families, many chemical substances were present in higher amounts in children than in their parents. When considering this in terms of the food that we eat and the food that we feed our children, farming techniques become of immediate concern. Due to the fuels used to run machinery, the pesticides used to protect crops, and the lack of mandatory field rotation in conventional farming methods, the levels of both heavy metals and essential metals in foods are marked concerns for consumers.

Considering the above questions, this study was formulated to achieve the following objectives:

1. To quantify levels of the heavy metal toxins cadmium, lead, and nickel in commercially available conventional and organic baby foods.
2. To quantify levels of the nutrient metals calcium, iron, and zinc in commercially available conventional and organic baby foods.
3. To compare the results concerning the metals in question between conventional and organic baby foods.
4. To determine whether or not there is a marked difference between conventional baby foods and those packaged as organic.

Two widely marketed and commercially available brands of baby food were chosen for analysis. Second stage green beans and carrots from the conventional and organic lines of food from each company were chosen for analysis by flame atomic absorption spectrophotometry. This subject was personal to me, as my husband and I have a young daughter and have often

wondered if the organic counterparts to conventional products are, in fact, worth the price difference.

CHAPTER 4

MATERIALS AND METHODS

Reagents Used

The reagents selected for use in this study were:

1. 1 000 ppm standard stock solutions of cadmium, calcium, iron, lead, nickel, and zinc, manufactured by Leeman Lab, Hudson, NH
2. Trace metal grade nitric acid manufactured by Seastar Chemical from Fisher Scientific, Pittsburgh, PA, lot # 1S92-2

Instruments Used

The primary instrument used was the Shimadzu AA 6300 model flame atomic absorption spectrophotometer manufactured by the Shimadzu Corporation of Japan. Hot plates manufactured by Fischer Scientific, Fair Lawn, NJ, were used in preparation of samples during the digestion and ashing process.

Lamp, Slit, and Wavelength Used

The atomic absorption method calls for the use of separate hollow cathode lamps specific to each element analyzed. Hollow cathode lamps for each metal in question manufactured by Perkin-Elmer Corporation, Norwalk, CT were used. The wavelengths and slits used were as recommended by the equipment manufacturer. Slits were 0.2 nm for iron and 0.7 nm for other metals examined. Wavelengths used were 228.8 nm for cadmium, 422.7 nm for calcium,

248.3 nm for iron, 232.0 nm for nickel, 289.3 nm for lead, and 213.9 nm for zinc.

Statistical Software Used

Microsoft Office Excel 2007 published by Microsoft Corporation, Redmond, WA was used to generate data spreadsheets, manipulate functions, and generate calibration curves. Microsoft Office Excel 2007 was also used to perform the ANOVA test for variance in terms of organic and conventional, as well as brand to brand comparisons.

Experimental Procedure

Sampling Method

Conventional and organic varieties of second stage green beans and carrots from two commercially available marketers were purchased from supermarkets in western North Carolina and eastern Tennessee. One container of each type of vegetable being tested was purchased together. Purchases were made at separate supermarkets to ensure that samples came from different lots.

The preparation of each sample began on the same day. Samples were taken from each container in triplicates. Upon opening, samples were skimmed from the surface and then taken from the middle and bottom of the container. Samples were staggered in this way to ensure that results would be representative of metal concentrations. Samples were labeled as shown in Table 1 so as to designate brand, farming and processing technique, the container sampled from, and where within the container the sample was taken.

Table 1. Select commercial samples and sample coding, labeling, and tracking

Sample Code	Sample Description
GCGB1A	Brand “G”; Conventional green beans; Container #1; Top skim
GCGB1B	Brand “G”; Conventional green beans; Container #1; Middle
GCGB1C	Brand “G”; Conventional green beans; Container #1; Bottom
BOGB2A	Brand “B”; Organic green beans; Container #2; Top skim
BOGB2B	Brand “B”; Organic green beans; Container #2; Middle
BOGB2C	Brand “B”; Organic green beans; Container #2; Bottom
GCC1A	Brand “G”; Conventional carrots; Container #1; Top skim
GCC1B	Brand “G”; Conventional carrots; Container #1; Middle
GCC1C	Brand “G”; Conventional carrots; Container #1; Bottom
BOC2A	Brand “B”; Organic carrots; Container #2; Top skim
BOC2B	Brand “B”; Organic carrots; Container #2; Middle
BOC2C	Brand “B”; Organic carrots; Container #2; Bottom

Sample Treatment

Samples were taken directly from their packaging and about 2.0 grams were weighed on an analytical balance. Table 2 shows the recorded mass of each sample.

Table 2. Measured Masses of Samples

Conventional Green Beans	Mass	Organic Green Beans	Mass	Conventional Carrots	Mass	Organic Carrots	Mass
GCGB1A	2.006	GOGB1A	2.039	GCC1A	2.084	GOC1A	2.055
GCGB1B	2.059	GOGB1B	2.080	GCC1B	2.009	GOC1B	2.059
GCGB1C	2.059	GOGB1C	2.044	GCC1C	2.008	GOC1C	2.015
GCGB2A	2.055	GOGB2A	2.052	GCC2A	2.068	GOC2A	2.024
GCGB2B	2.016	GOGB2B	2.034	GCC2B	2.08	GOC2B	2.019
GCGB2C	2.014	GOGB2C	2.042	GCC2C	2.018	GOC2C	2.052
BCGB1A	2.021	BOGB1A	2.032	BCC1A	2.068	BOC1A	2.051
BCGB1B	2.041	BOGB1B	2.049	BCC1B	2.004	BOC1B	2.031
BCGB1C	2.068	BOGB1C	2.024	BCC1C	2.060	BOC1C	2.016
BCGB2A	2.059	BOGB2A	2.087	BCC2A	2.063	BOC2A	2.057
BCGB2B	2.047	BOGB2B	2.020	BCC2B	2.033	BOC2B	2.097
BCGB2C	2.079	BOGB2C	2.015	BCC2C	2.035	BOC2C	2.045

Each sample was massed in triplicates, for a total of 48 samples for two containers each of conventional and organic varieties of green beans and carrots from two leading baby food producers. All samples were placed directly into 100-mL beakers in preparation for digestion. Upon completion of weighing of samples, 30 mL of concentrated trace metal grade nitric acid were added to each beaker under a fume hood, and the samples were allowed to stand for approximately 18 hours. The majority of the organic material was able to dissolve during the standing period, with each solution turning a yellow-green color. Carrots seemed to dissolve more completely than green beans. The organic varieties of both vegetables had the most un-dissolved residue that settled to the bottom of the beaker. Each sample was then slowly heated on a hot plate with a glass stir rod in place to avoid any bumping during the heating process. Upon heating, each sample turned an orange brown color. Green bean samples developed an initial murkiness that cleared as the samples began to boil. Each sample was heated to near dryness, leaving small amounts of residue in each. The heating process took approximately three hours. Beakers containing the sample residue were allowed to cool before the addition of approximately 10 mL of de-ionized water to re-dissolve the residue for quantitative transfer and dilution. Samples were filtered using a vacuum filtration system with Whatman No.2, 42.5 mm filter paper. Beakers containing samples were rinsed at least three times with de-ionized water during the filtration process. Filtered solutions were quantitatively transferred to 50-mL volumetric flasks and diluted to the mark. Each sample was then transferred to clean, pre-labeled plastic containers to avoid adsorption of metals to the glass container. The blank was also prepared in triplicates by the same process using the same amount of nitric acid with no sample present. Blanks were also transferred to plastic containers for storage. Prepared blanks and samples were refrigerated until analysis.

Preparation of Standard Solutions

All standard solutions used for calibration were prepared by serial dilution of the 1000 ppm stock solutions. Standard solutions of concentration 0.05, 0.10, 0.20, 0.50, 1.00, and 2.00 ppm were prepared immediately before sample analysis to avoid fluctuations in concentration due to adsorption or decomposition. Fresh standards were prepared for reproducibility and recovery studies.

Determination of Metal Contents in Baby Food Samples by Atomic Absorption Technique

Flame atomic absorption spectrophotometry was used to analyze samples of conventional and organic baby foods for cadmium, calcium, iron, lead, nickel, and zinc. Due to the mechanism of analysis, each metal was analyzed for separately. Standard solutions for individual metals were run using the respective hollow cathode lamps to generate calibration curves of the different metals. Samples were run through the instrument in the same way. The standard addition method was used to examine the effects of interferents in the sample solutions. Selected samples were checked for cadmium and iron by this method. Recovery studies were done by first placing one-mL aliquots of each sample in 25-mL volumetric flasks. Standard solutions were added and diluted to the mark so as to make additions of 0, 0.20, and 0.40 ppm standards to the corresponding sample aliquots. All of the solutions were then run through the flame AA and the concentrations of the metals in them were determined.

Statistics Used

To determine concentration distributions among samples the mean, median, standard deviation, and average deviation were calculated. Data collected were also studied based on a

95% confidence interval, and an ANOVA analysis for variance was done to compare organic versus conventional and brand G versus brand B. All statistical analysis calculations were performed using Microsoft Excel.

CHAPTER 5

RESULTS AND DISCUSSION

The goal of this research project was to compare two leading baby food brands in terms of their conventional and organic varieties. Conventional and organic varieties of stage two green beans and carrots produced by two different companies were purchased from different grocery stores in both North Carolina and Tennessee in hopes of getting a more accurate picture of average content of the metals being examined. Stage two foods were used because they are of a thicker consistency and tend to be the bridge between baby foods and table foods for many children. As such, children seem to be fed this stage of foods for a longer period of time than the other stages available.

Metal Concentrations Between Brands Tested

Comparisons were made based on concentrations. The significance of the differences between both organic and conventional varieties of green beans and carrots, as well as differences between brands, were determined using variation studies calculated via ANOVA. Two way ANOVA tables may be found in Appendices C and D. As a whole, the results obtained did not show organic varieties of the vegetables tested to be better than their conventionally grown and processed counterparts. Significant differences seen between organic and conventional varieties often pointed to conventional foods as the better choice. Differences were more apparent between samples from brand G and those from brand B.

Cadmium

Overall, cadmium would not be considered a threatening contaminant of any of the foods tested. However, extremely small amounts were detected in several samples of both green beans and carrots.

For the green beans tested, no cadmium was detected in either brand of the conventional food. Limited amounts were detected in 3 of the 12 organic brand samples tested, with two samples from brand G and one sample from brand B being found to contain concentrations less than 0.03 ppm, as shown in Table 3.

Table 3. Measured Concentrations of Cadmium in Green Beans. Shown below are the results obtained using the calibration curve method for cadmium levels in micrograms per gram of sample (µg / g) for each sample tested.

	Brand G Conventional	Brand G Organic	Brand B Conventional	Brand B Organic
1A	0.000	0.014	0.000	0.000
1B	0.000	0.026	0.000	0.000
1C	0.000	0.000	0.000	0.000
2A	0.000	0.000	0.000	0.000
2B	0.000	0.000	0.000	0.000
2C	0.000	0.000	0.000	0.027

According to the ANOVA analysis, there was a significant difference between the organic and conventional green beans tested ($p > 0.05$). However, it should be noted that because cadmium was detected only sporadically in the sample group of organic green beans, it is unlikely that this heavy metal be viewed as a threat here. It is possible that these values are due to tiny residual amounts present in the atmosphere or precipitation, as these levels would most likely fluctuate from one location to the next, with green beans being more susceptible to this type of deposition due to the edible portion of the plant growing above ground.

The results for cadmium in carrots were in contrast to those of green beans in that the metal was detected only in the conventional varieties, albeit in extremely small amounts.

Table 4. Measured Concentrations of Cadmium in Carrots. Shown below are the results obtained using the calibration curve method for cadmium levels in micrograms per gram of sample (µg / g) for each sample tested.

	Brand G Conventional	Brand G Organic	Brand B Conventional	Brand B Organic
1A	0.000	0.000	0.000	0.000
1B	0.008	0.000	0.000	0.000
1C	0.000	0.000	0.000	0.000
2A	0.000	0.000	0.001	0.000
2B	0.000	0.000	0.000	0.000
2C	0.000	0.000	0.000	0.000

As shown in Table 4, cadmium was found to be present in only 2 of 12 conventional samples tested, with concentrations of only 0.008 ppm and 0.001 ppm, far lower than those found in the organic green beans. Statistical analysis showed there to be a significant difference between conventional and organic ($p > 0.05$), though the same consideration made with the green beans should be taken here. Again, the sporadic detection of cadmium in the carrot samples tested does not show the metal to be a threat in these foods. With only 2 of 12 samples showing detectable levels of cadmium, both of which were conventional carrots, the results here do not conclusively indicate organic farming techniques to be better. It seems that carrots would be good indicators in this argument, as cadmium is taken up through plant roots. The edible portion of the carrot is the root portion that grows beneath the soil surface, making it susceptible to deposition of all varieties.

Although farm location with regards to pollution sources could explain the presence of cadmium deposition and absorption, it is also possible that cadmium deposition could occur as a result of the conventional farming techniques. Cadmium can be a by-product in the exhaust

generated by the burning of fossil fuels. This would mean cadmium should be found in greater abundance in carrots than green beans, as the edible portion of the plant is grown below the soil surface, thereby making it more subject to higher levels of soil contaminants.

Statistical analysis shows brand B to be the better choice for either green beans or carrots. However, the significant difference could come from the relatively small sample set. Of 24 samples from each brand, 3 brand G samples and 2 brand B samples showed cadmium to be present. Of the total 48 samples, cadmium was present in only 5. Perhaps more samples would change the effect of these few outliers.

Cadmium does not seem to be a concern overall due to its inconsistent detecting and low level presence in both conventional and organic samples of both carrots and green beans. It can therefore be stated here that the baby foods marketed as organic do not present advantages over those marketed as conventional in regards to this heavy metal.

Lead

Lead is commonly deposited in particulate form from the burning of fossil fuels. A more likely contaminant than cadmium, lead levels were examined in an effort to study the effects of farming techniques. Although lead was detected in both green beans and carrots, none of the levels detected should be of concern, even for children, as all were below the FDA maximum of 0.1 ppm [20].

In the green bean samples tested, lead levels were most prominent in the conventional varieties of brand G, with five of the six samples having detectable concentrations and an overall mean concentration of 0.050 ppm. Conventional varieties of brand B showed detectable levels for four of the six samples, though the levels were generally lower than those for brand G, with a

mean concentration of only 0.020 ppm. Both organic varieties showed lower concentrations of lead than the conventional varieties, with the presence of lead being more sporadic as well. Organic green beans from brand G showed detectable concentrations in only two of six samples, and in only one of six samples of brand B. Table 5 shows the measured concentrations found in each sample. Mean concentrations for organic brands G and B were 0.005 ppm and 0.003 ppm, respectively. Statistical analysis also showed a significant difference among samples, with organic green beans having significantly lower lead values.

Table 5. Measured Concentrations of Lead in Green Beans. Shown below are the results obtained using the calibration curve method for lead levels in micrograms per gram of sample (µg / g) for each sample tested.

	Brand G Conventional	Brand G Organic	Brand B Conventional	Brand B Organic
1A	0.023	0.023	0.008	0.000
1B	0.097	0.000	0.030	0.000
1C	0.052	0.000	0.000	0.015
2A	0.067	0.000	0.000	0.000
2B	0.060	0.000	0.030	0.000
2C	0.000	0.008	0.052	0.000

The results for carrots are given in Table 6 and showed more overall samples with detectable levels of lead than green beans, which may be due to carrots being grown below the soil surface. In contrast to the green beans, lead was found more often in the organic varieties of carrots. Lead was detected in only 2 of the 6 samples of brand G conventional carrots and in 3 of the 6 samples of brand B conventional carrots. The mean values for lead in conventional carrots were 0.015 ppm and 0.008 ppm for brands G and B, respectively. Lead was detected in 4 of the 6 samples of brand G organic carrots and in 5 of the 6 samples of brand B organic carrots.

Organic varieties showed mean values of 0.040 ppm and 0.042 ppm for brands G and B, respectively.

Table 6. Measured Concentrations of Lead in Carrots. Shown below are the results obtained using the calibration curve method for lead levels in micrograms per gram of sample (µg / g) for each sample tested.

	Brand G Conventional	Brand G Organic	Brand B Conventional	Brand B Organic
1A	0.045	0.001	0.008	0.065
1B	0.045	0.047	0.000	0.067
1C	0.000	0.000	0.015	0.009
2A	0.000	0.021	0.023	0.069
2B	0.000	0.000	0.000	0.000
2C	0.000	0.171	0.000	0.039

Statistically, conventional varieties of carrots showed significantly lower levels of lead.

The lead measurements among the organic varieties seem inconsistent in terms of the range covered. Measurements were rechecked during the experiment using the same samples. New samples were not created here in order to preserve the conditions of the study. However, in further research it would be necessary to look more closely at lead levels in both conventional and organic samples to see if results differ from those obtained here.

Interestingly, lead was detected more often and at higher levels in the organic brands tested. Theoretically lead deposition should be less of a concern for organic farms, as fuels and fertilizers or other topical treatments that could directly deposit the contaminant are not permissible. Furthermore, any organic farm must have a documented waiting period between any prior conventional farming and the onset of organic farming [21].

Upon researching each company, it was found that although both brands of baby food were marketed nationally, brand G obtained its vegetables from widespread farms, while brand B

obtained vegetables either from its own growing grounds or from farms in much closer proximity.

Lead was detected in small amounts across all sample groups tested. However, each sample series contained samples in which no lead was detected. Both brands of organic carrots showed more samples with detectable levels of lead than either conventional sample series. In any case, all concentrations detected were well below the FDA regulation of 0.100 ppm. Again, the results obtained do not hold to the claims made concerning organically grown and processed foods. Organic vegetables should have consistently lower levels of contaminants, such as lead or cadmium, whose primary source of deposition would be from the use of fuels, fertilizers, or pesticides used only in conventional farming practices. There was also not a significant difference apparent between brands tested ($p < 0.05$), which was in contrast to other results.

Nickel

The most likely pathway for nickel to reach crops is via the burning of fuels or through the use of fertilizers or other topical agents applied to conventionally farmed crops. It would seem that conventionally grown crops would be more susceptible to this form of deposition, as conventional methods employ equipment that runs on gas or diesel fuels.

Table 7 shows the measured concentrations of nickel in green beans. Of those tested, only the conventionally grown brand B samples showed no nickel present. However, detectable levels of nickel were found in only one sample of the brand B organic green beans. The conventional green beans sampled from brand G showed a mean nickel concentration of 0.025 ppm, with nickel being detected in 5 of the 6 samples prepared. The organic variety of brand G showed a mean concentration of 0.007 ppm, with nickel being detected in only half of the

prepared samples. Nickel was detected in only 1 of the 6 prepared samples for brand B organic green beans at a concentration of 0.011 ppm, giving the sample series a mean of 0.002 ppm.

Table 7. Measured Concentrations of Nickel in Green Beans. Shown below are the results obtained using the calibration curve method for nickel levels in micrograms per gram of sample (µg / g) for each sample tested.

	Brand G Conventional	Brand G Organic	Brand B Conventional	Brand B Organic
1A	0.009	0.031	0.000	0.000
1B	0.078	0.000	0.000	0.011
1C	0.006	0.007	0.000	0.000
2A	0.000	0.006	0.000	0.000
2B	0.048	0.000	0.000	0.000
2C	0.011	0.000	0.000	0.000

When comparing organic versus conventional, statistical studies showed there to be no significant difference between the two ($p < 0.05$). However, it should be noted that only conventional green beans from brand B had a completely clear sample series. The significant difference was apparent in brand comparison, with brand B showing much better results than brand G overall.

As mentioned earlier, brand B uses vegetables that are grown on their own farms or on farms in relatively close proximity. As such, it follows that levels of metals would be fairly consistent between both the organic and conventional varieties, as the environmental conditions of growing sites would be more similar. Brand G, on the other hand, acquires vegetables from farms that are geographically much more widespread. Farms in different regions of the United States would be more subject to different environmental conditions in terms of pollution deposition due to wind or weather patterns, uses of fertilizers, pesticides, and other topical agents, industrial proximity, etc. This seems to be the case as detectable levels of nickel were

found in almost every brand G sample, although the levels were well below the levels of concern issued by the FDA and EPA of 0.100 ppm.

In keeping with results above, nickel was detected only sporadically among the samples of carrots tested, though here organic methods seemed better. The results concerning nickel in carrot samples is shown in Table 8. No nickel was detected in either brand of organic carrots, while only small quantities (<0.08 ppm) were detected in two of the six samples of each brand of conventional carrots.

Table 8. Measured Concentrations of Nickel in Carrots. Shown below are the results obtained using the calibration curve method for lead levels in micrograms per gram of sample (µg / g) for each sample tested.

	Brand G Conventional	Brand G Organic	Brand B Conventional	Brand B Organic
1A	0.012	0.000	0.000	0.000
1B	0.000	0.000	0.000	0.000
1C	0.016	0.000	0.000	0.000
2A	0.000	0.000	0.047	0.000
2B	0.000	0.000	0.000	0.000
2C	0.000	0.000	0.011	0.000

The presence of nickel in conventional samples is more likely, as it is this methodology that would employ the use of topical agents that may contain trace amounts of nickel.

Although the organic varieties showed no detectable levels of nickel, this still would not point to organic being better than conventional. Levels of nickel detected were sporadic among the conventional samples, and all were below the FDA and EPA maximums of 0.100 ppm, well below concentrations that would be considered alarming. Furthermore, statistical studies showed the difference between organic and conventional carrots to be insignificant.

Iron

An essential nutrient, especially for growing children, iron levels were examined as a means of studying the effects of farming techniques on the levels of metal ions that should be present in food. One of the claims made in terms of organically grown vegetables over those grown using conventional farming methods is the prevention of nutrient loss [1]. Some of the chemicals used in conventional techniques, particularly pesticides and fertilizers, have the ability to leech nutrients, such as iron, from the roots of plants.

Like other dark green varieties of vegetables, green beans are a common source of dietary iron. Iron levels detected were higher in organic varieties of brand G, but in conventional varieties of brand B. Table 9 shows the results for iron in green beans for each sample.

Table 9. Measured Concentrations of Iron in Green Beans. Shown below are the results obtained using the calibration curve method for iron levels in micrograms per gram of sample (μg / g) for each sample tested.

	Brand G Conventional	Brand G Organic	Brand B Conventional	Brand B Organic
1A	0.085	0.483	0.051	0.000
1B	0.094	0.088	0.057	0.000
1C	0.136	0.143	0.028	0.000
2A	0.191	0.020	0.083	0.008
2B	0.248	0.275	0.000	0.000
2C	0.202	0.242	0.878	0.000

In comparing the conventional brands tested, brand G showed a mean iron concentration of 0.159 ppm, while brand B was slightly higher with a mean of 0.183 ppm. Iron levels were therefore reasonably consistent between the conventional varieties, and would be a satisfactory contributor to the 11 mg per day recommended by the American Academy of Pediatrics when considered in relation to other dietary contributors across daily meals. Iron levels were found to

be slightly higher in the samples from organic brand G than either conventional variety, with a mean of 0.209 ppm. However, samples analyzed from organic brand B showed a mean concentration of only 0.001 ppm. The results obtained for organic brand B were far lower than organic brand G or the conventional samples tested from either brand.

The results obtained for brand B were interesting in that the conventional variety showed significantly higher levels of iron than the organic variety, as evidenced in Table 9 above. Upon further inspection, it was found that brand G conventional green beans tested were fortified with iron during industrial processing.

As expected, iron levels found in carrots were generally lower than those found in green beans, as green beans are a better source of dietary iron. The results did show that conventional carrots from brand B contained the highest concentrations of iron, while the concentrations found in organic carrots of brand B were lowest, as was the case with green beans. The results concerning iron concentration in carrot samples are shown in Table 10.

Table 10. Measured Concentrations of Iron in Carrots. Shown below are the results obtained using the calibration curve method for iron levels in micrograms per gram of sample (µg / g) for each sample tested.

	Brand G Conventional	Brand G Organic	Brand B Conventional	Brand B Organic
1A	0.000	0.000	0.000	0.120
1B	0.183	0.000	0.026	0.006
1C	0.000	0.000	0.000	0.000
2A	0.000	0.000	0.662	0.155
2B	0.047	0.389	0.088	0.000
2C	0.108	0.110	0.016	0.000

In comparing results, the mean values were examined as an initial indicator. Conventional brand B carrots showed the highest mean concentration at 0.132 ppm. Brand G

samples had mean concentrations of 0.083 ppm for the organic carrots and 0.056 for the conventional carrots. Organic carrots sampled from brand B showed the lowest mean concentration at 0.047 ppm. The measurements taken for organic samples from brand G and conventional samples from brand B were relatively inconsistent, with one major outlier in each set. Again, new samples were not prepared in order to preserve study conditions. However, in future studies this would be something to reexamine.

Calcium

As with iron, calcium levels were examined in order to study effects of farming techniques on the levels of essential nutrients. Both conventional and organic varieties of green beans and carrots tested presented levels of calcium, an important nutrient needed for growth and development in babies and young children, that would be deemed acceptable by the FDA or the American Association of Pediatrics. It was initially expected that the organic varieties of each vegetable would contain higher levels of nutrients due to the leeching of nutrients that can happen to crops grown under conventional methods. The results obtained for calcium do not necessarily place organic as being better than conventional.

The results for green beans were interesting due to unexpected relationships between the organic and conventional brands. The conventional varieties produced results that aligned with one another. However, the results for the organic green beans did not align between brands G and B, as can be seen in Table 11.

Table 11. Measured Concentrations of Calcium in Green Beans. Shown below are the results obtained using the calibration curve method for calcium levels in micrograms per gram of sample (µg / g) for each sample tested.

	Brand G Conventional	Brand G Organic	Brand B Conventional	Brand B Organic
1A	23.564	2.601	24.277	13.621
1B	27.183	19.885	22.867	14.062
1C	23.847	38.420	22.671	12.223
2A	24.184	45.282	24.804	14.563
2B	25.691	38.899	20.484	13.017
2C	27.629	39.770	24.891	12.783

Statistically, no significant difference was found between conventional and organic varieties when compared overall. The mean for brand G conventional samples was 25.350 ppm, slightly higher than that for brand B at 23.332 ppm. The organic variety of brand G green beans showed higher concentrations of calcium, with a mean of 30.810 ppm. When considering only these results, it would seem that organic green beans appear to be better, thus following the assertions that the pesticides, herbicides, and fertilizers applied to conventionally grown crops deplete nutrients. However, the organic green beans from brand B showed concentration of calcium much lower than those found in either of the conventional brands, with a mean of only 13.378 ppm. It was for this reason that a significant difference was seen in calcium levels between brands G and B. All readings obtained in the organic brand B sample series were close to one another, so the mean was not lowered by a single outlier measurement. This raises questions about the location of farms and the quality of soil in which vegetables are being grown, as the lower levels here cannot be blamed upon nutrient depletion due to topical agents.

The results for carrots are given in Table 12 and showed higher calcium concentrations among the conventional samples. Both conventional brands and both organic brands aligned with one another as would be expected, unlike the results for the green beans tested. The means

for conventional brands G and B were found to be 16.583 ppm and 16.434 ppm, respectively. The means for organic brands G and B were 13.105 ppm and 13.597 ppm, respectively. In terms of organic versus conventional, means for each growing method correlated with one another. However, the conventional brands showed higher concentrations of calcium, without fortification.

Table 12. Measured Concentrations of Calcium in Carrots. Shown below are the results obtained using the calibration curve method for calcium levels in micrograms per gram of sample (μg / g) for each sample tested.

	Brand G Conventional	Brand G Organic	Brand B Conventional	Brand B Organic
1A	16.255	13.469	18.296	12.359
1B	15.439	13.115	15.923	13.926
1C	17.061	12.805	11.390	12.157
2A	18.127	13.387	18.938	15.945
2B	16.658	12.217	17.218	15.270
2C	15.956	13.638	16.837	11.923

A significant difference was apparent between conventional and organic carrots, with higher levels present overall among the conventional varieties tested. There was no real significant difference from one brand to the next in terms of calcium.

Zinc

Like iron and calcium, zinc was included in the study in order to monitor the effects of farming techniques on essential nutrients. The results for zinc continue to support the overall findings that, in terms of baby foods tested, there is no true benefit in choosing organic over conventional. Both green bean samples and carrot samples presented reasonably consistent levels of zinc in samples across the board.

For the green bean samples, no brand or variety stood out in terms of results obtained. Table 13 shows the measured concentration obtained for each sample. Results seemed to hover between 0.030 ppm and 0.058 ppm, with the exception of a few outliers. Mean values calculated for conventional brands were 0.048 ppm and 0.059 ppm for brand G and brand B. The measurements taken for brand B did have one significant outlier, which served to raise the mean. For organic brands tested the mean values were 0.0388 ppm for brand G and 0.056 ppm for brand B.

Table 13. Measured Concentrations of Zinc in Green Beans. Shown below are the results obtained using the calibration curve method for zinc levels in micrograms per gram of sample (µg / g) for each sample tested.

	Brand G Conventional	Brand G Organic	Brand B Conventional	Brand B Organic
1A	0.054	0.054	0.024	0.054
1B	0.054	0.003	0.053	0.054
1C	0.046	0.040	0.072	0.070
2A	0.058	0.038	0.054	0.040
2B	0.038	0.058	0.102	0.053
2C	0.036	0.040	0.046	0.065

The strongest correlation here was between brands rather than a farming technique correlation. This would suggest, as with other results obtained for metals studied, that environment and surroundings play an important role in metal content of crops.

Carrots did not produce results that were any more conclusive in terms of organic versus regular than those given by green beans. At least one sample in each series showed no detectable levels of zinc, which was surprising as carrots tend to be a good source of this micronutrient. The results for zinc in carrots are shown in Table 14.

Table 14. Measured Concentrations of Zinc in Carrots. Shown below are the results obtained using the calibration curve method for zinc levels in micrograms per gram of sample (µg / g) for each sample tested.

	Brand G Conventional	Brand G Organic	Brand B Conventional	Brand B Organic
1A	0.000	0.033	0.033	0.000
1B	0.000	0.033	0.033	0.000
1C	0.018	0.072	0.058	0.013
2A	0.000	0.000	0.000	0.023
2B	0.023	0.003	0.000	0.033
2C	0.000	0.033	0.023	0.000

Zinc concentrations were highest for the samples taken from brand G organic carrots, with a mean of 0.029 ppm. In contrast, carrots from organic brand B showed zinc contents less than half those of brand G, with a mean of 0.012 ppm. Conventional carrots sampled from brand B showed a mean zinc concentration of 0.025 ppm, while brand G showed a mean of only 0.007 ppm. Due to the differences between organic brands, it cannot be clearly stated that organic carrots in general are a better source of zinc than conventional varieties.

Linearity

Calibration curves were generated using standard solutions of 0.05, 0.10, 0.20, 0.50, and 1.00 ppm for each metal in question, as per recommended protocol. An example is given in Figure 1. All other calibration curves used in this study may be found in Appendix A.

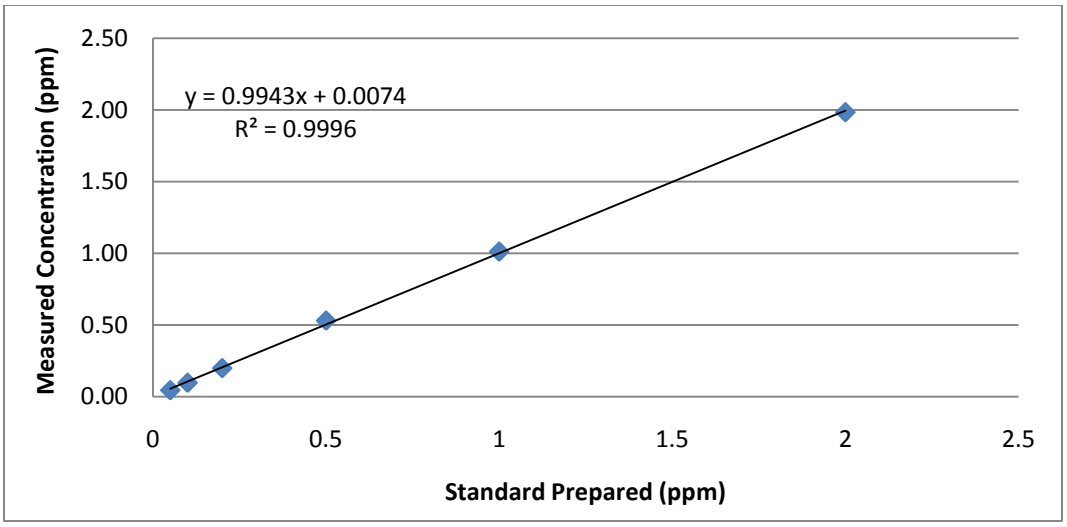

Figure 1. Standard Calibration Curve for Iron: Concentrations of metals in the sample solutions were determined with the help of calibration curves of this nature.

The calibration curve shown in Figure 1, and others found in Appendix A, show a high degree of linearity, thus illustrating there to be little experimental error. All curves were made using standard concentrations above the detection limits for each metal.

Interferences and Recovery Studies

When conducting chemical analyses by the use of flame atomic absorption, there are generally two factors to consider in terms of accuracy and precision. Any instrument may show small differences in the data collected when used at different times. This instrument factor may be overcome by running both the standards and the sample solutions in the same setting. All samples were analyzed immediately following the measurement of standards for the calibration curve. The instrument was not turned off between the running of designated metal standards and sample sets being analyzed for said metal.

A more troublesome factor that may affect analysis by the use of flame atomic absorption is that of matrix interference. According to several analytical texts, matrix interference is seen

generally as the primary problem associated with this form of chemical analysis [25, 26, 27]. Matrix interferences presents most often in the normal calibration curve method, as the chemical environments of the standard and sample solutions differ. Standards are prepared from pure substances, conceivably containing only the metal ion in question. The sample solutions are not formulated from pure substances and may contain a laundry list of interfering compounds naturally present and / or formed during the treatment and digestion processes [29]. To determine whether or not such interferences were present here, the standard addition method was employed to analyze some samples for iron and lead. An example of the standard addition calibration curve used for the determination of metal concentration is shown in Figure 2.

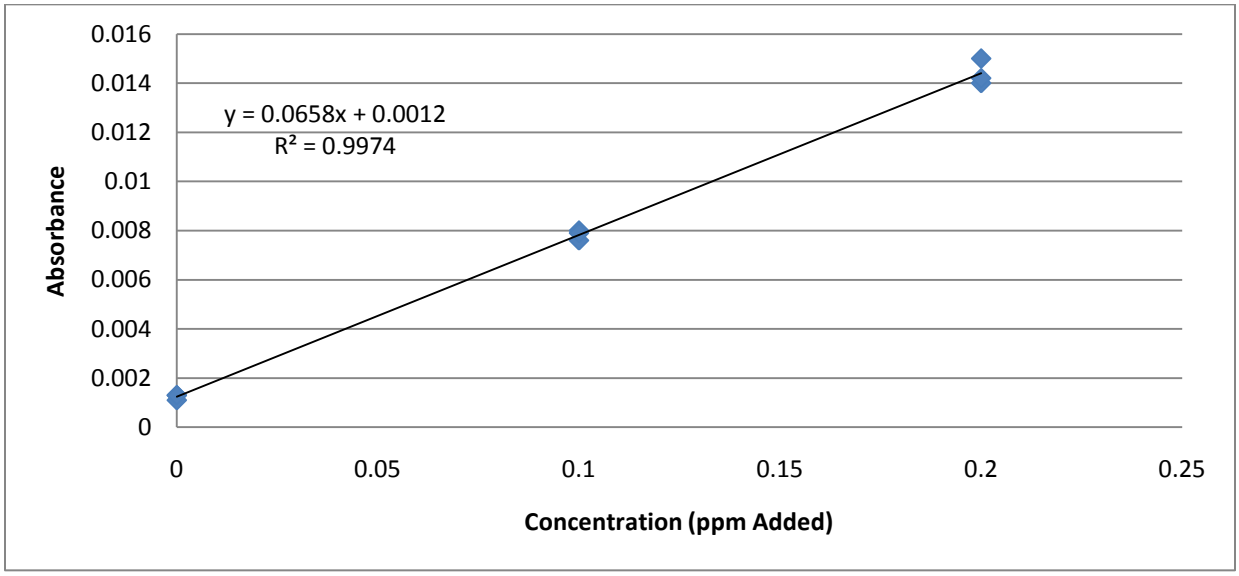

Figure 2. Determination of Metal Concentration by Standard Addition for Iron: The standard addition method can determine metal concentrations present in the sample solution while minimizing matrix interferences.

A comparison of concentrations of each metal found by the normal calibration curve method and the standard addition method are shown in Tables 15 and 16.

Table 15. Comparison of the Concentration of Iron in Sample Solutions As Determined by Calibration Curve and Standard Addition Methods: Concentrations are expressed as ppm.

G-CGB	Calibration Curve Concentration	Standard Addition Concentration	Difference	% Difference
1A	0.085	0.094	-0.0091	-10.8
1B	0.094	0.091	0.0032	3.5
1C	0.136	0.094	0.0414	30.5

Table 16. Comparison of the Concentration of Lead in Sample Solutions As Determined by Calibration Curve and Standard Addition Methods: Concentrations are expressed as ppm.

G-CGB	Calibration Curve Concentration	Standard Addition Concentration	Difference	% Difference
1A	0.001	0.037	-0.037	-12170
1B	0.047	0.037	0.010	21.4
1C	0.040	0.043	-0.003	-6.3

The data show that some interferences are present for some metals in some samples. Standard addition methods yielded results that were generally lower than those of the calibration curve method for iron, while the opposite seems to be true for lead. As such, iron is subject to negative interferences while lead is subject to positive interferences. In both cases, the A samples seemed to deviate from the other two. It should be noted that these samples were created by skimming from the top of the containers, which could impact the amount of heavier metal ions present in the sample.

To evaluate the data obtained via the flame atomic absorption spectrophotometer, recovery studies were also performed. Recovery studies were performed on iron in brand "G" conventional green beans and on lead in brand "G" organic carrots. As stated earlier in the discussion of the standard addition method, three aliquots of samples were used to prepare solutions in triplicates. The first solution was prepared without addition, the second solution

with a 0.10 ppm addition of standard metal solution, and the third solution with a 0.20 ppm addition of standard metal solution. Data obtained from the recovery studies are displayed in Tables 17 and 18.

Table 17. Recovery Study for Iron from Sample G-CGB: The first aliquot has not addition, thus the table shows zero recovery. The second aliquot has 0.1 ppm addition. The third aliquot has 0.2 ppm addition. Values shown are averages based on triplicate samples.

Addition (ppm)	Absorbance	Concentration (ppm)	Recovery	% Recovery
0	0.0012	0.0931	NA	NA
0.10	0.0078	0.195	1.019	101.9 %
0.20	0.0144	0.296	1.016	101.6 %

Table 18. Recovery Study for Lead from Sample G-OC: The first aliquot has not addition, thus the table shows zero recovery. The second aliquot has 0.1 ppm addition. The third aliquot has 0.2 ppm addition. Values shown are averages based on triplicate samples.

Addition (ppm)	Absorbance	Concentration (ppm)	Recovery	% Recovery
0	0.0025	0.039	NA	NA
0.10	0.0040	0.127	0.879	87.9 %
0.20	0.0054	0.215	0.879	87.9 %

From the results shown in Table 17, the recovery for iron in Brand G conventional bean samples were very good in that there seems to be little if any interference present at all. For the Brand G organic carrot sample, however, the recoveries for lead were about 88.0%. While the values were not as desirable, however, they were within experimental errors considering that lead tends to have worse detection limits and thus relatively larger errors may occur. So the data obtained for this study of comparing the conventional and organic varieties of baby foods from different brands, and to observe if there is statistically significant difference can be found

between the two types were valid. This is also the case because all results obtained would have similar errors and they would cancel out and would not affect the comparisons.

CHAPTER 6

CONCLUSION

This study sought to determine whether or not organic baby food was advantageous over conventional baby food in terms of both contaminant heavy metals and essential nutrient metals. According to the results obtained, there is no alarming difference between the organic and the conventional foods marketed by the brands tested even though statistical difference was shown for certain metals in certain brands of baby foods. In general, there is a slight advantage of organic baby foods over the conventional ones in terms of the slightly elevated amounts of undesirable metals in the conventional foods. However, the amount found was low and sporadic among the samples studies. Although this study was performed on only green beans and carrots, the results could be expanded to include most vegetables, as those tested include a plant whose edible portion grew above ground and one whose edible portion grew beneath the surface. This allowed the study to potentially cover means of common deposition of contaminants or of common removal of nutrients.

This study was born of personal interest, as it began out of my own questions surrounding the options available to parents when it comes to baby food brands and types. Though it is a certainty that the debate over organic and conventional foods is far from settled, it is relieving to see that this study provided no reason to prefer organic varieties of vegetables. Due to the nature of the claims made in terms of organic foods, it was expected that results would be quite different between organically grown and processed foods and conventionally grown and processed foods. However, in no way did this study show organic baby foods to be consistently better than conventional foods. The results for iron, calcium, and zinc did not point to

conventional methods causing significant nutrient loss. Likewise, the results for the heavy metal ions cadmium, lead, and nickel did not show conventional techniques as infusing crops with toxins. However, when considering baby foods, the growing process alone cannot be scrutinized, as the vegetables are cleaned, cooked, and processed in a factory before reaching a child's plate.

Future Direction

The debate concerning the choice of organic products over their conventional counterparts is one that will be present for some time. Therefore, studies surrounding this debate should continue as well. As vegetables are generally considered to be valid environmental receptors, studies such as this can produce a reliable picture of the farming techniques employed and regulations followed.

The results here seem to point to environmental factors outside the farming techniques employed as having a possibly significant impact on metals present in vegetables. Though it would again require a working relationship with the companies involved, it would be interesting to study the locations of farms where crops originated. The more widespread the farms, the greater the differences in terms of industrial proximity, weather and wind patterns, chemical agents used by farmers, irrigation sources, and other factors that could contribute to varying levels of chemicals in foods.

In terms of nationally marketed brands such as those used in this study, it would be interesting to examine their regulations and quality control measures more closely. This would require a working relationship with the companies themselves in order to better understand measures taken to ensure quality and would help complete the picture of organic baby food

versus conventional baby food by showing how the farming and the processing procedures fit together.

It would be interesting to conduct further studies of this nature on foods that are marketed by smaller companies. More and more organic baby food companies are emerging, with many having roots as small organizations that were initially run out of a residential kitchen. These companies generally purchase their vegetables from small growers, comparable in size to their own business. It would be interesting to see if there was a difference between the results obtained for large companies, such as those in this study, and smaller operations.

In terms of expanding the research, it would be of interest to include more vegetables, as well as varieties of fruits in the study. It seems that companies would be more likely to import various fruits from outside the United States, which could allow for varying levels of metals to be apparent.

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APPENDIXES

APPENDIX A

Calibration Curve for Various Metals

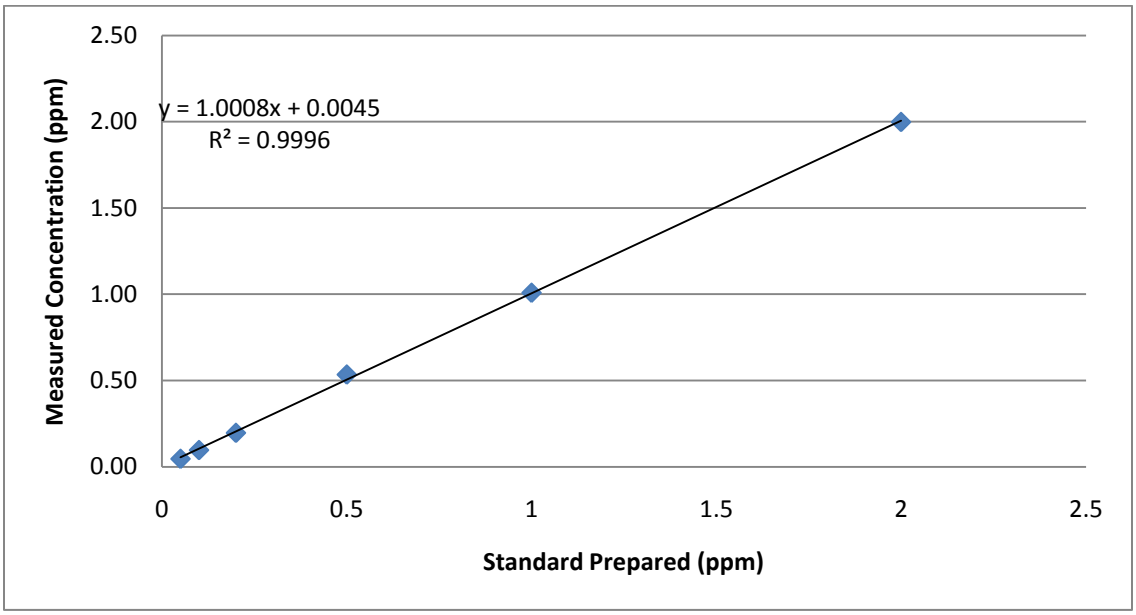

Figure 3. Calibration Curve for Cadmium

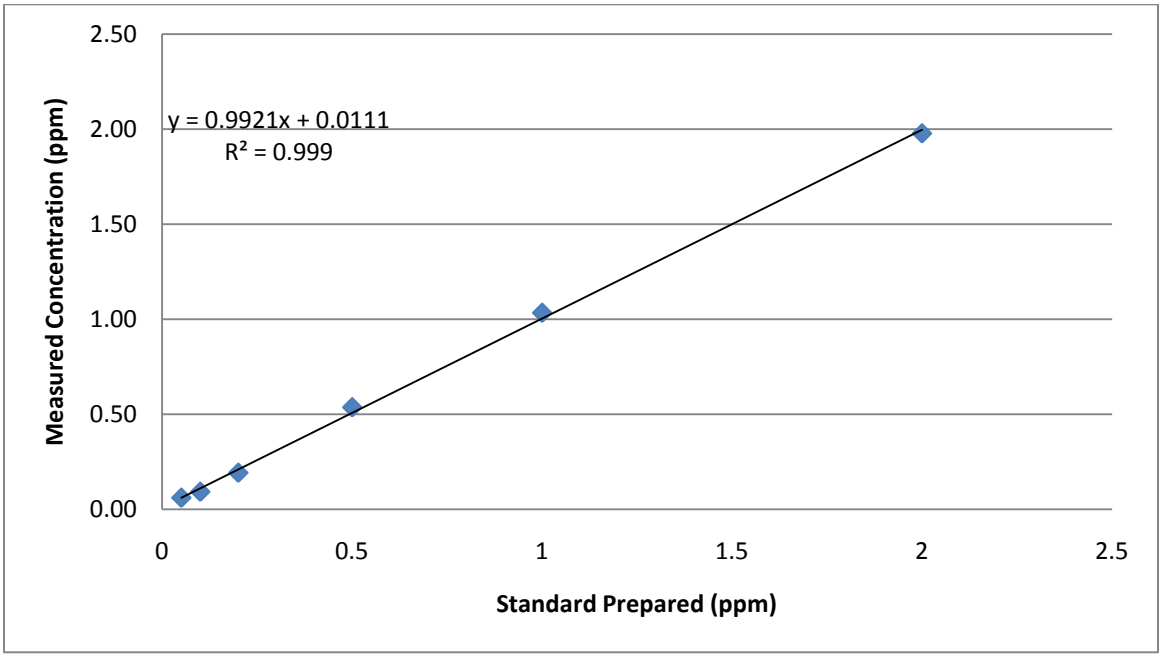

Figure 4. Calibration Curve for Lead

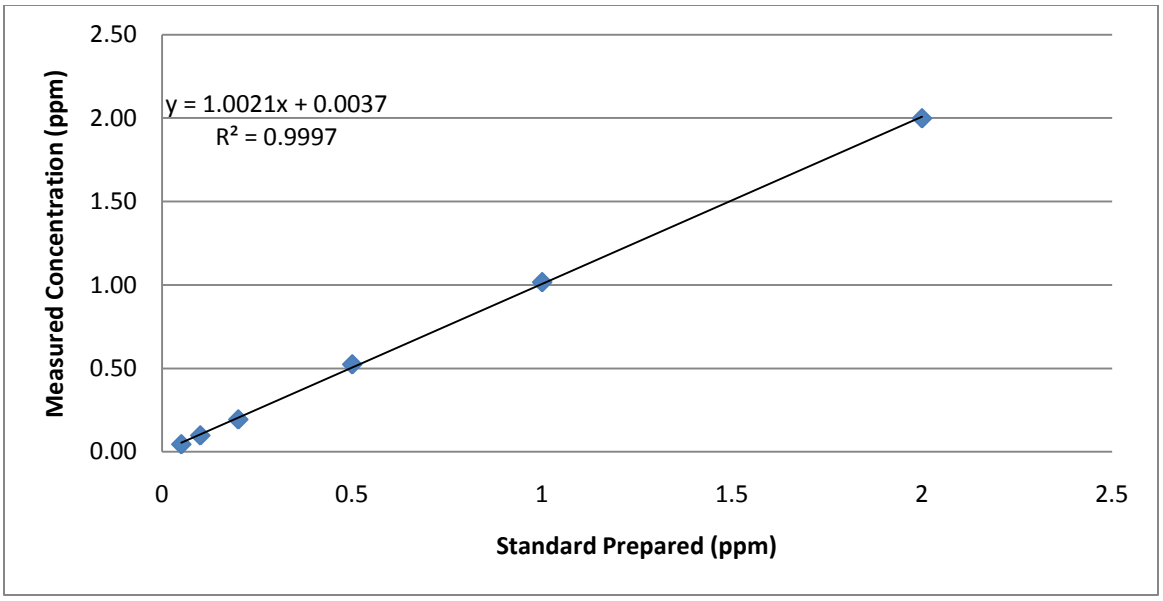

Figure 5. Calibration Curve for Nickel

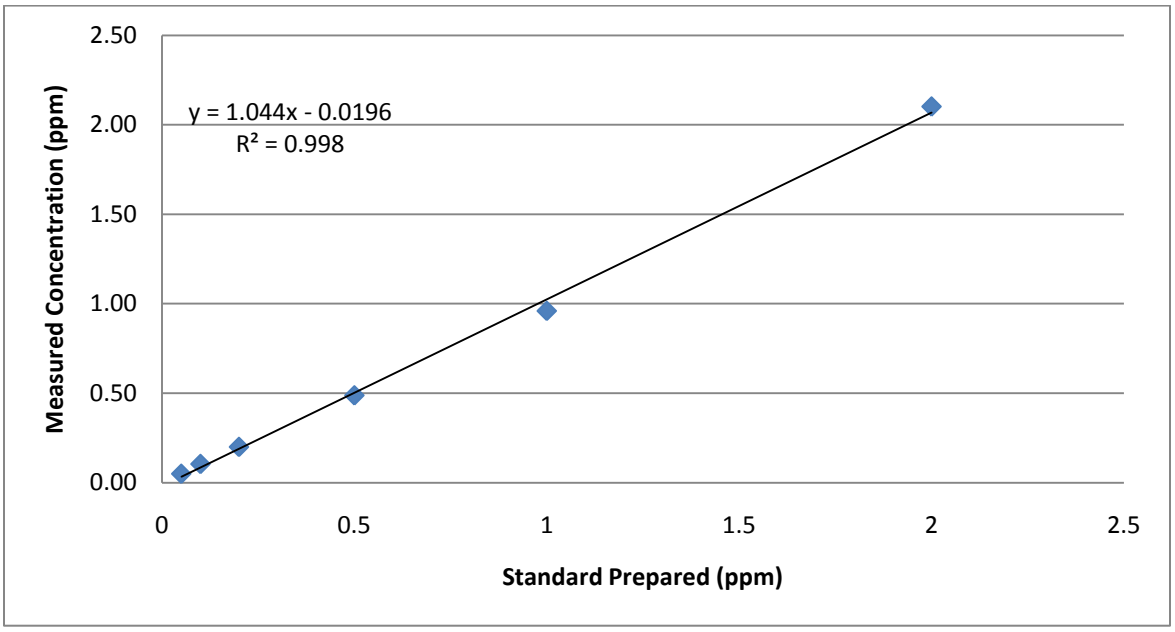

Figure 6. Calibration Curve for Calcium

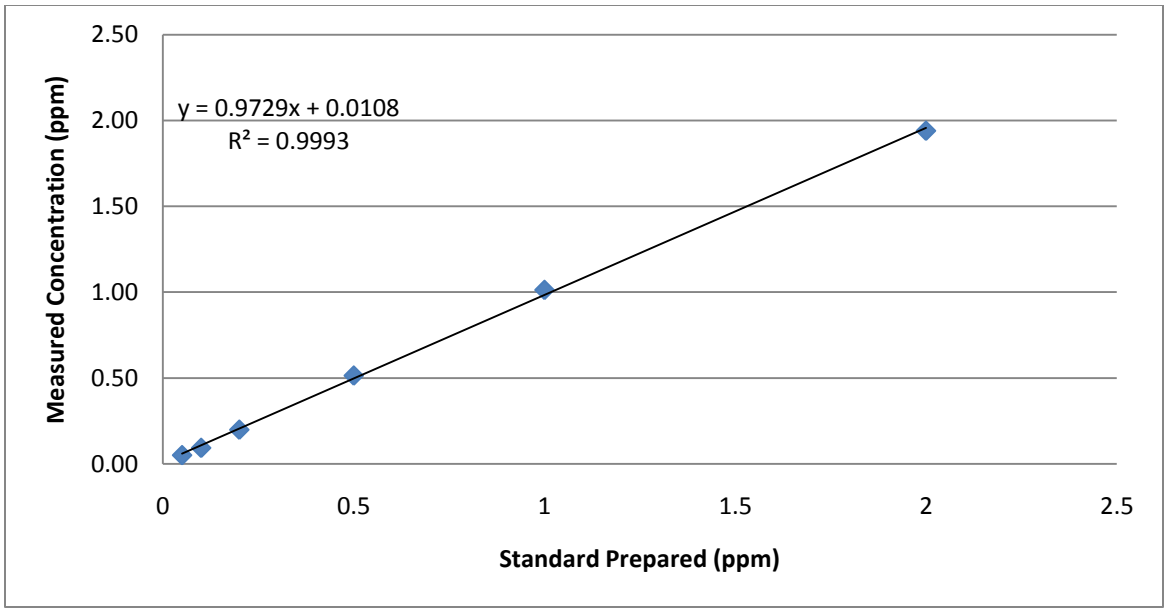

Figure 7. Calibration Curve for Zinc

APPENDIX B

Statistical Summary Tables

Table 19. Statistical Values for Cadmium in Green Beans

				95% Conf. Limit		Value		
Sample Code	Mean	Median	Stand. Dev.	Lower	Higher	Min	Max	Avg. Dev.
G-CGB	0.0000	0.0000	0.0000	NA	NA	0.0000	0.0000	0.0000
G-OGB	0.0066	0.0000	0.0109	0.0000	0.0153	0.0000	0.0260	0.0088
B-CGB	0.0000	0.0000	0.0000	NA	NA	0.0000	0.0000	0.0000
B-OGB	0.0045	0.0000	0.0109	0.0000	0.0133	0.0000	0.0270	0.0074

Table 20. Statistical Values for Cadmium in Carrots

				95% Conf. Limit		Value		
Sample Code	Mean	Median	Stand. Dev.	Lower	Higher	Min	Max	Avg. Dev.
G-CC	0.0014	0.0000	0.0033	0.0000	0.0040	0.0000	0.0080	0.0023
G-OC	0.0000	0.0000	0.0000	NA	NA	0.0000	0.0000	0.0000
B-CC	0.0001	0.0000	0.0002	0.0000	0.0003	0.0000	0.0010	0.0002
B-OC	0.0000	0.0000	0.0000	NA	NA	0.0000	0.0000	0.0000

Table 21. Statistical Values for Lead in Green Beans

				95% Conf. Limit		Value		
Sample Code	Mean	Median	Stand. Dev.	Lower	Higher	Min	Max	Avg. Dev.
G-CGB	0.0499	0.0560	0.0343	0.0224	0.0774	0.0000	0.0970	0.0258
G-OGB	0.0050	0.0000	0.0091	0.0000	0.0122	0.0000	0.0230	0.0067
B-CGB	0.0199	0.0190	0.0209	0.0031	0.0367	0.0000	0.0520	0.0174
B-OGB	0.0025	0.0000	0.0061	0.0000	0.0074	0.0000	0.0150	0.0042

Table 22. Statistical Values for Lead in Carrots

				95% Conf. Limit		Value		
Sample Code	Mean	Median	Stand. Dev.	Lower	Higher	Min	Max	Avg. Dev.
G-CC	0.1497	0.0000	0.0232	0.0000	0.0336	0.0000	0.045	0.0120
G-OC	0.0468	0.0310	0.0637	0.0000	0.0977	0.0000	0.1710	0.0413
B-CC	0.0075	0.0040	0.0095	0.0000	0.0151	0.0080	0.0150	0.0075
B-OC	0.0415	0.0520	0.0306	0.0170	0.0660	0.0000	0.0690	0.0254

Table 23. Statistical Values for Nickel in Green Beans

				95% Conf. Limit		Value		
Sample Code	Mean	Median	Stand. Dev.	Lower	Higher	Min	Max	Avg. Dev.
G-CGB	0.0252	0.0100	0.0310	0.0004	0.0500	0.0000	0.0780	0.0252
G-OGB	0.0073	0.0030	0.0120	0.0000	0.0169	0.0000	0.0310	0.0067
B-CGB	0.0000	0.0000	0.0000	NA	NA	0.0000	0.0000	0.0000
B-OGB	0.0018	0.0000	0.0044	0.0000	0.0053	0.0000	0.0110	0.0030

Table 24. Statistical Values for Nickel in Carrots

				95% Conf. Limit		Value		
Sample Code	Mean	Median	Stand. Dev.	Lower	Higher	Min	Max	Avg. Dev.
G-CC	0.0047	0.0000	0.0073	0.0000	0.0106	0.0000	0.0160	0.0062
G-OC	0.0000	0.0000	0.0000	NA	NA	0.0000	0.0000	0.0000
B-CC	0.0096	0.0000	0.0189	0.0000	0.0251	0.0000	0.0470	0.0129
B-OC	0.0000	0.0000	0.0000	NA	NA	0.0000	0.0000	0.0000

Table 25. Statistical Values for Iron in Green Beans

				95% Conf. Limit		Value		
Sample Code	Mean	Median	Stand. Dev.	Lower	Higher	Min	Max	Avg. Dev.
G-CGB	0.1592	0.1630	0.0647	0.1074	0.2110	0.2040	0.3670	0.0543
G-OGB	0.2085	0.1930	0.1645	0.0769	0.3401	0.1390	0.6020	0.1247
B-CGB	0.1827	0.0540	0.3418	0.0000	0.4558	0.0000	0.8780	0.2318
B-OGB	0.0013	0.0000	0.0032	0.0000	0.0038	0.0000	0.0080	0.0022

Table 26. Statistical Values for Iron in Carrots

				95% Conf. Limit		Value		
Sample Code	Mean	Median	Stand. Dev.	Lower	Higher	Min	Max	Avg. Dev.
G-CC	0.0563	0.0240	0.0751	0.0000	0.1164	0.0000	0.1830	0.0593
G-OC	0.0832	0.0000	0.1561	0.0000	0.2081	0.0000	0.3890	0.1109
B-CC	0.1319	0.0210	0.2617	0.0000	0.3413	0.0000	0.6620	0.1767
B-OC	0.0468	0.0030	0.0711	0.0000	0.1037	0.0000	0.1550	0.0604

Table 27. Statistical Values for Calcium in Green Beans

				95% Conf. Limit		Value		
Sample Code	Mean	Median	Stand. Dev.	Lower	Higher	Min	Max	Avg. Dev.
G-CGB	25.35	24.94	1.759	23.94	26.76	23.56	27.63	1.485
G-OGB	34.14	38.660	10.33	25.88	42.40	21.03	46.43	8.600
B-CGB	23.33	23.57	1.689	21.98	24.68	20.48	24.89	1.325
B-OGB	13.38	13.32	0.8660	12.69	14.07	12.22	14.56	0.7038

Table 28. Statistical Values for Calcium in Carrots

				95% Conf. Limit		Value		
Sample Code	Mean	Median	Stand. Dev.	Lower	Higher	Min	Max	Avg. Dev.
G-CC	16.58	16.46	0.9411	15.83	17.33	15.44	18.13	0.6993
G-OC	13.11	13.25	0.5242	12.69	13.53	12.22	13.64	0.3961
B-CC	16.43	17.03	2.691	14.28	18.58	11.39	18.94	1.851
B-OC	13.60	13.14	1.722	12.22	14.98	11.92	15.95	1.450

Table 29. Statistical Values for Zinc in Green Beans

				95% Conf. Limit		Value		
Sample Code	Mean	Median	Stand. Dev.	Lower	Higher	Min	Max	Avg. Dev.
G-CGB	0.0476	0.0500	0.0090	0.0404	0.0548	0.0360	0.0580	0.0076
G-OGB	0.0388	0.0400	0.0193	0.0234	0.0542	0.0030	0.0580	0.0121
B-CGB	0.0588	0.0540	0.0265	0.0376	0.0800	0.0240	0.102	0.0191
B-OGB	0.0559	0.0540	0.0106	0.0474	0.0644	0.0400	0.070	0.0077

Table 30. Statistical Values for Zinc in Carrots

				95% Conf. Limit		Value		
Sample Code	Mean	Median	Stand. Dev.	Lower	Higher	Min	Max	Avg. Dev.
G-CC	0.0068	0.0000	0.0107	0.0000	0.0154	0.0000	0.0230	0.0091
G-OC	0.0292	0.0330	0.0263	0.0081	0.0503	0.0000	0.0720	0.0186
B-CC	0.0246	0.0280	0.0223	0.0068	0.0424	0.0000	0.0580	0.0170
B-OC	0.0116	0.0070	0.0142	0.0002	0.0230	0.0000	0.0330	0.0112

APPENDIX C

Two Way ANOVA Tables for Organic Versus Conventional

Table 31. Two Way ANOVA Table for Cadmium in Green Beans

Comparison of Organic Versus Conventional

Source of Variation	Sum of Squares	Df	Mean Square	F	P-value	F-crit
Sample	1.846E-04	1	1.846E-04	3.118	0.093	4.351
Columns	6.242E-06	1	6.242E-06	0.105	0.749	4.351
Interaction	6.242E-06	1	6.242E-06	0.105	0.749	4.351
Within	1.184E-03	20	5.920E-05			
Total	1.381E-03	23				

Table 32. Two Way ANOVA Table for Cadmium in Carrots

Comparison of Organic Versus Conventional

Source of Variation	Sum of Squares	Df	Mean Square	F	P-value	F-crit
Sample	3.154E-06	1	3.153E-06	1.147	0.297	4.351
Columns	2.344E-06	1	2.344E-06	0.853	0.367	4.351
Interaction	2.344E-06	1	2.344E-06	0.853	0.367	4.351
Within	5.500E-05	20	2.749E-06			
Total	6.282E-05	23				

Table 33. Two Way ANOVA Table for Lead in Green Beans

Comparison of Organic Versus Conventional

Source of Variation	Sum of Squares	Df	Mean Square	F	P-value	F-crit
Sample	5.825E-03	1	5.825E-03	13.385	1.561E-03	4.351
Columns	1.589E-03	1	1.589E-03	3.652	7.045E-02	4.351
Interaction	1.139E-03	1	1.139E-03	2.616	0.121	4.351
Within	8.704E-03	20	4.352E-04			
Total	1.726E-02	23				

Table 34. Two Way ANOVA Table for Lead in Carrots

Comparison of Organic Versus Conventional

Source of Variation	Sum of Squares	Df	Mean Square	F	P-value	F-crit
Sample	6.494E-03	1	6.494E-03	4.623	0.044	4.351
Columns	2.419E-04	1	2.419E-04	0.172	0.683	4.351
Interaction	7.482E-06	1	7.482E-06	5.326E-03	0.943	4.351
Within	2.810E-02	20	1.405E-03			
Total	3.484E-02	23				

Table 35. Two Way ANOVA Table for Nickel in Green Beans

Comparison of Organic Versus Conventional

Source of Variation	Sum of Squares	Df	Mean Square	F	P-value	F-crit
Sample	3.890E-04	1	3.890E-04	1.381	0.254	4.351
Columns	1.414E-03	1	1.414E-03	5.021	0.037	4.351
Interaction	5.804E-04	1	5.804E-04	2.061	0.167	4.351
Within	5.632E-03	20	2.816E-04			
Total	8.015E-03	23				

Table 36. Two Way ANOVA Table for Nickel in Carrots

Comparison of Organic Versus Conventional

Source of Variation	Sum of Squares	Df	Mean Square	F	P-value	F-crit
Sample	3.080E-04	1	3.080E-04	3.004	0.098	4.351
Columns	3.693E-05	1	3.693E-05	0.360	0.555	4.351
Interaction	3.693E-05	1	3.693E-05	0.360	0.555	4.351
Within	2.050E-03	20	1.025E-04			
Total	2.432E-03	23				

Table 37. Two Way ANOVA Table for Iron in Green Beans

Comparison of Organic Versus Conventional

Source of Variation	Sum of Squares	Df	Mean Square	F	P-value	F-crit
Sample	0.026	1	0.026	0.706	0.411	4.351
Columns	0.051	1	0.051	1.368	0.256	4.351
Interaction	0.080	1	0.080	2.157	0.157	4.351
Within	0.740	20	0.037			
Total	0.897	23				

Table 38. Two Way ANOVA Table for Iron in Carrots

Comparison of Organic Versus Conventional

Source of Variation	Sum of Squares	Df	Mean Square	F	P-value	F-crit
Sample	5.100E-03	1	5.100E-03	0.197	0.662	4.351
Columns	2.315E-03	1	2.315E-03	0.089	0.768	4.351
Interaction	1.881E-02	1	1.881E-02	0.727	0.404	4.351
Within	0.518	20	2.589E-02			
Total	0.544	23				

Table 39. Two Way ANOVA Table for Calcium in Green Beans

Comparison of Organic Versus Conventional

Source of Variation	Sum of Squares	Df	Mean Square	F	P-value	F-crit
Sample	2.021	1	2.021	0.071	0.792	4.351
Columns	778.522	1	778.522	27.478	3.951E-05	4.351
Interaction	527.207	1	527.207	18.608	3.379E-04	4.351
Within	566.644	20	28.332			
Total	1874.395	23				

Table 40. Two Way ANOVA Table for Calcium in Carrots

Comparison of Organic Versus Conventional

Source of Variation	Sum of Squares	Df	Mean Square	F	P-value	F-crit
Sample	59.809	1	59.809	21.042	1.786E-04	4.351
Columns	0.176	1	0.176	0.062	0.806	4.351
Interaction	0.615	1	0.615	0.216	0.647	4.351
Within	56.847	20	2.842			
Total	117.448	23				

Table 41. Two Way ANOVA Table for Zinc in Green Beans

Comparison of Organic Versus Conventional

Source of Variation	Sum of Squares	Df	Mean Square	F	P-value	F-crit
Sample	2.041E-04	1	2.041E-04	0.645	0.431	4.351
Columns	1.196E-03	1	1.196E-03	3.777	0.066	4.351
Interaction	5.400E-05	1	5.400E-05	0.171	0.684	4.351
Within	6.332E-03	20	3.166E-04			
Total	7.786E-03	23				

Table 42. Two Way ANOVA Table for Zinc in Carrots

Comparison of Organic Versus Conventional

Source of Variation	Sum of Squares	Df	Mean Square	F	P-value	F-crit
Sample	1.307E-04	1	1.307E-04	0.347	0.563	4.351
Columns	2.667E-08	1	2.667E-08	7.077E-05	0.993	4.351
Interaction	1.887E-03	1	1.887E-03	5.008	0.0368	4.351
Within	7.536E-03	20	3.768E-04			
Total	9.553E-03	23				

APPENDIX D

Two Way ANOVA Tables for Brand G Versus Brand B

Table 43. Two Way ANOVA Table for Cadmium in Green Beans

Brand Comparison

Source of Variation	Sum of Squares	Df	Mean Square	F	P-value	F-crit
Sample	6.242E-06	1	6.242E-06	0.105	0.749	4.351
Columns	1.846E-04	1	1.846E-04	3.118	0.093	4.351
Interaction	6.242E-06	1	6.242E-06	0.105	0.749	4.351
Within	1.184E-03	20	5.920E-05			
Total	1.381E-03	23				

Table 44. Two Way ANOVA Table for Cadmium in Carrots

Brand Comparison

Source of Variation	Sum of Squares	Df	Mean Square	F	P-value	F-crit
Sample	2.344E-06	1	2.344E-06	0.853	0.367	4.351
Columns	3.154E-06	1	3.154E-06	1.147	0.297	4.351
Interaction	2.344E-06	1	2.344E-06	0.853	0.367	4.351
Within	5.498E-05	20	2.749E-06			
Total	6.282E-05	23				

Table 45. Two Way ANOVA Table for Lead in Green Beans

Brand Comparison

Source of Variation	Sum of Squares	Df	Mean Square	F	P-value	F-crit
Sample	0.002	1	0.002	3.652	0.070	4.351
Columns	0.006	1	0.006	13.385	0.002	4.351
Interaction	0.001	1	0.001	2.616	0.121	4.351
Within	0.009	20				
Total	0.017	23				

Table 46. Two Way ANOVA Table for Lead in Carrots

Brand Comparison

Source of Variation	Sum of Squares	Df	Mean Square	F	P-value	F-crit
Sample	2.419E-04	1	2.419E-04	0.172	0.683	4.351
Columns	6.494E-03	1	6.494E-03	4.623	0.044	4.351
Interaction	7.482E-06	1	7.482E-06	5.326E-03	0.943	4.351
Within	0.028	20	1.405E-03			
Total	0.035	23				

Table 47. Two Way ANOVA Table for Nickel in Green Beans

Brand Comparison

Source of Variation	Sum of Squares	Df	Mean Square	F	P-value	F-crit
Sample	1.414E-03	1	1.414E-03	5.021	0.037	4.351
Columns	3.890E-04	1	3.890E-04	1.381	0.254	4.351
Interaction	5.804E-04	1	5.804E-04	2.061	0.167	4.351
Within	5.632E-03	20	2.816E-04			
Total	0.008	23				

Table 48. Two Way ANOVA Table for Nickel in Carrots

Brand Comparison

Source of Variation	Sum of Squares	Df	Mean Square	F	P-value	F-crit
Sample	3.693E-05	1	3.693E-05	0.360	0.555	4.351
Columns	3.080E-04	1	3.080E-04	3.004	0.098	4.351
Interaction	3.693E-05	1	3.693E-05	0.360	0.360	4.351
Within	0.002	20	1.025E-04			
Total	0.002	23				

Table 49. Two Way ANOVA Table for Iron in Green Beans

Brand Comparison

Source of Variation	Sum of Squares	Df	Mean Square	F	P-value	F-crit
Sample	0.051	1	0.051	1.368	0.256	4.351
Columns	0.026	1	0.026	0.706	0.411	4.351
Interaction	0.080	1	0.080	2.157	0.157	4.351
Within	0.740	20	0.037			
Total	0.897	23				

Table 50. Two Way ANOVA Table for Iron in Carrots

Brand Comparison

Source of Variation	Sum of Squares	Df	Mean Square	F	P-value	F-crit
Sample	0.002	1	0.002	0.089	0.768	4.351
Columns	0.005	1	0.005	0.197	0.662	4.351
Interaction	0.019	1	0.019	0.727	0.404	4.351
Within	0.518	20	0.026			
Total	0.544	23				

Table 51. Two Way ANOVA Table for Calcium in Green Beans

Brand Comparison

Source of Variation	Sum of Squares	Df	Mean Square	F	P-value	F-crit
Sample	778.522	1	778.522	27.478	3.951E-05	4.351
Columns	2.021	1	2.021	0.071	0.792	4.351
Interaction	527.207	1	527.207	18.608	3.379E-04	4.351
Within	566.645	20	28.332			
Total	1874.395	23				

Table 52. Two Way ANOVA Table for Calcium in Carrots

Brand Comparison

Source of Variation	Sum of Squares	Df	Mean Square	F	P-value	F-crit
Sample	0.176	1	0.176	0.062	0.806	4.351
Columns	59.809	1	59.809	21.042	1.786E-04	4.351
Interaction	0.615	1	0.615	0.216	0.647	4.351
Within	56.847	20	2.842			
Total	117.448	23				

Table 53. Two Way ANOVA Table for Zinc in Green Beans

Brand Comparison

Source of Variation	Sum of Squares	Df	Mean Square	F	P-value	F-crit
Sample	1.196E-03	1	1.196E-03	3.777	0.066	4.351
Columns	2.042E-04	1	2.042E-04	0.645	0.431	4.351
Interaction	5.400E-05	1	5.400E-05	0.171	0.684	4.351
Within	6.332E-03	20	3.166E-04			
Total	7.786E-03	23				

Table 54. Two Way ANOVA Table for Zinc in Carrots

Brand Comparison

Source of Variation	Sum of Squares	Df	Mean Square	F	P-value	F-crit
Sample	2.667E-08	1	2.667E-08	7.077E-05	0.993	4.351
Columns	1.307E-04	1	1.307E-04	0.347	0.563	4.351
Interaction	1.887E-03	1	1.887E-03	5.008	0.037	4.351
Within	7.536E-03	20	3.768E-04			
Total	9.553E-03	23				

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