5-2023

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Recommended Citation

David Garuba, Omobolanle Oluwafemi, "Evaluation and Heavy Metal Concentration Assessment in Selected Brands of Infant and Toddler Food Types in Houston, Texas" (2023). *Theses (2016-Present)*. 51.
https://digitalscholarship.tsu.edu/theses/51

EVALUATION AND HEAVY METAL CONCENTRATION ASSESSMENT IN SELECTED BRANDS OF INFANT AND TODDLER FOOD TYPES IN HOUSTON, TEXAS

THESIS

Presented in Partial Fulfillment of the Requirements for
the Degree Master of Science in the Graduate School
of Texas Southern University

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2023

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By

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Heavy metals and metalloids such as lead, cadmium, arsenic, nickel and zinc are an unavoidable contaminant of our ecosystem because of its natural occurrence They are also introduced into the environment anthropogenically through manufacturing, industrial agricultural processes which contaminates the food chain when taken up by food producing crops as a result of their presence in soil and water used in planting or feeding livestock. The adverse effects of these toxic elements have become a global threat to food security, particularly due to their inextricable association with human health. Exposure to environmental contaminants from daily diet is a major concern for all ages, although children are more vulnerable to their effects because they consume more food relative to their body weight and have under developed nervous system. Exposure to toxic metals in children presents long-term health risks to growing infants and toddlers and has been linked but not limited to a variety of health issues such as disruptive behavior, neurological

damage and attention deficit hyperactivity. A report recently released by the U.S House of Representatives in 2021, raised concerns about the dangerously elevated levels of heavy metals in baby food due to improper testing of raw ingredients used in making baby foods and finished baby food products and under-reporting by food manufacturers, of these high levels of toxicity which keeps toxic products on the market. Although heavy metals can be found in some foods due to contaminated water and soil, their levels in foods, especially baby foods should be of great concern. The U.S. Food and Drug Administration (FDA) has proposed daily permissible limits for some of these metals; however, a major challenge to this remains subpar testing practices, lenient self-regulating standards set by different food manufacturers where there are inconsistent and conflicting tolerable safe limit values set by various food agencies.

The new U.S. FDA initiative, known as Closer to Zero Plan (C2Z) seeks to reduce to almost zero level, the toxic element exposures from foods eaten by babies and young children, therefore, this study was aimed at evaluating heavy metal concentrations in baby and toddler food products targeted at infant and toddler age groups produced by leading brands indicated in the report. Ten commercial baby foods from the top seven leading brands in the United States were purchased from a local store, representing six ingredient categories listed as a diary, fruit; leguminous vegetable; beef, chicken, root vegetable; or grains and evaluated for arsenic (As), cadmium (Cd), Zinc (Zn) lead (Pb), Nickel (Ni), aluminum (Al) and chromium (Cr) in triplicates for heavy metal concentrations using the triple quadrupole inductively coupled mass spectrometry) QQQ_ICP-MS), a tandem mass spectrometer method that has the capability of detecting analytes at lower detection limits.

Nickel, Chromium and Zinc are vital in living organisms and necessary for metabolic and immune support while Arsenic, and Lead are non-essential but can be highly toxic even at extremely low concentrations of 0.005ug/g. In all the food types evaluated, aluminum (4.09 µg/g and 2.50 µg/g) and zinc (33.5 µg/g 69.5 µg/g, and 30.2 µg/g) were the most elevated in the infant food age group while lead and cadmium metals in all other food types were observed at levels not exceeding the tolerable limits except in rice cereal. The acceptable daily limits of Aluminum and Zinc are 1 µg/g /day and 0.3 µg/g /day. The mixed model generated for this analysis found significant differences in metal concentrations ($F_{6,24}$=2.75, p=0.03). The overall average metal concentration in the food was 0.96 µg/g. No significant correlations were found between the packaging materials used and the observed metal concentrations (P >0.05, Std error 1.94**).** Food products formulated from fruits and root tubers commonly referred to as plant-based food products, exhibited the highest concentrations of all tested heavy metals although none of the food labels reported the levels of heavy metal concentrations in the food products. Transparency in reporting toxic metal content on food labels will help consumers make an informed decision when purchasing these food products.

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LIST OF ABBREVIATIONS

WHO	World Health Organization
NCDs	Non communicable diseases
Et al	et alia
Zn	Zinc
Al	Aluminum
Cd	Cadmium
Cr	Chromium
As	Arsenic
Pb	Lead
Ni	Nickel
FDA	Food and Drug Administration
EPA	Environmental Protection Agency
ATSDR	Agency for Toxic Substances and Disease Registry
g/cm3	Gram per centimeter cube
ug/g	Microgram per gram
PPM	Parts per million
AAS	Atomic absorption spectrometry
ICP-MS	Inductively coupled mass spectrometry
ICP-OES	Inductively coupled optical emission spectrometry
QQQ-ICP-MS	Triple quadrupole inductively coupled mass spectrometry
SD	Standard deviation

UNICEF	United Nation’s Child Fund
DNA	Deoxyribonucleic acid
iAS	Inorganic arsenate
MMA	Monommethylarsonic acid
DMA	Dimethylarsonic acid
Cr2+	Divalent chromium
Cr6+	Hexavalent chromium
Cr3+	Trivalent chromium
ADHD	Attention deficit hyperactivity disorder
HBBF	Healthy Babies, Bright futures
C2Z	Closer to zero

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ACKNOWLEDGEMENTS

I give all thanks to God through my Lord Jesus Christ, for helping me to accomplish this task. It seemed like a never-ending journey and is the longest I have ever taken in pursuit of a degree, but I am thankful for the strength and grace to complete it finally.

My sincere appreciation goes to my advisor, Dr. Ayodotun Sodipe, for believing in me and pushing me to make this a success. I appreciate all the constructive criticism, the help and the role he played in my life at this time. Thank you so much sir, I will never forget all I have learned from you. I also appreciate Dr. Sonya Good, Dr. Audrey Player and Dr. Shishir Shishodia for accepting to be members of my thesis committee and for every positive input given to make this research a success. I sincerely appreciate Dr. Shodimu-Emmanuel Olufemi for his input, help, concern and encouragement throughout this period of study. I am thankful to all the Professors in my department, whose well of intelligence impacted my academic learning in one way or the other. I sincerely thank my friends Kenny Joshua, Mr. Akinola Olatunde and Ms. Sekinat Atobiloye, for providing help, guidance and support throughout the study period.

My profound gratitude goes to my mom, Mrs. Felicia Adewuni Alabi who never stopped encouraging and praying for me every step of the way. Her prayers and support are the reasons I made this through. Thank you, mom, for being the best always! To my siblings, Mrs. Ekundayo Olalere, Mrs. Onaolapo Adeolu-Shittu, Bishop Oluwaponmile and Mrs. Bunmi Alabi, Mr. and Mrs. Abayomi Alabi who gave me all the support and encouragement I needed especially when I felt like giving up, I say a big thank you to you all for believing in me. I could never wish for another family.

I unreservedly appreciate my husband, Olukayode David Garuba who stood by me throughout this study, and my precious children, Stephen Olusegun and Susan Omobola, my two wonderful darlings, who understood in their own way every time I said I was busy, thank you for being the blessings in this journey I will always be thankful for.

CHAPTER 1

INTRODUCTION

Baby foods provide nourishment to a part of the human population that is easily vulnerable to illnesses and toxins, therefore, baby food manufacturers are usually extra vigilant with regards to the processing and quality parameters that are employed during production. During food processing involving cooking and heat treatments, several substances are formed as by-products. Some of these formed substances may add taste, color and texture to the food while helping to minimize harmful germs. Toxic substances and elements such as cadmium, arsenic, lead and mercury are often found in food, including baby foods as a result of their absorption by crops from soil and water during the planting period, or through the packaging materials used although research has shown that these are usually within the acceptable tolerable levels with minimal damage (Farag, 2020).

A recent evaluation of four major baby food companies presented by the subcommittee on Economic and Consumer Policy Committee on Oversight and Reform by the U.S. House of Representatives in 2021, indicated that baby foods are tainted with concerning dangerous levels of some heavy metals such as arsenic, lead, cadmium and mercury (Oversight.house.gov). According to the report, baby food manufacturers negligently produced these baby food products due to their subpar testing, lenient standards employed in properly testing for heavy metals in baby foods and sole reliance on primary testing of raw ingredients without the need for testing finished products for these metals.

These factors have consistently led to underestimation of heavy metal levels in finished food products. Studies have reported that contamination of selected infant formula milk by substances such as nitrates, aluminum, cadmium, mercury, nickel and lead are mostly from water sources which are used to reconstitute most of these baby foods (Ogochukwu et al., 2019).

Children's growth and development depends largely on the quality of food that is eaten and when these different food items are introduced to them. Therefore, the World Health Organization (WHO), recommends that infants should be exclusively breast fed for the first six months of life and thereafter be introduced to foods that act complementarily to support their growth and development. A wide variety of such food products recommended for infants and young children are formulated from a wide variety of fruits, cereals, meats and vegetables. To maintain their quality, baby foods are subjected to different strong thermal treatments that ensures microbiological safety, while enhancing their desirable sensory properties such as desired flavors and colors, digestibility safety, texture features as well as the elimination of enzymatic activities (Prata et al., 2021). Coincidentally, neo-contaminants derived from raw materials or during processing in infant foods may introduce potential toxic elements into these food products (Farag et al., 2020).

According to the WHO, a healthy diet helps to protect against not just malnutrition, but also noncommunicable diseases (NCDs) such as diabetes, heart disease, cancer, and its likes (WHO, 2022). Since healthy dietary practices start early in life, monitoring children's diet is an important aspect, contributing to general wellness. Although exclusive breastfeeding promotes and improves cognitive development, not every child benefits from this. Many babies start off on infant formulas due to demanding unavoidable situations such as maternal death, premature delivery, low birth weight and in most cases, the convenience of usage since any caregiver can help feed a baby as opposed to just the mother, especially due to the demands of work (Ibrahim et al., 2022). Consequently, as infants grow into the stage of complementary feeding, unhealthy diet habits lead to global health risks. Increased production of processed foods, rapid urbanization and changing lifestyles, therefore lead to a shift in children's dietary patterns, leading to consumption of foods that are high in energy, fats, sugars, salt/sodium and in most cases, heavy metals (WHO, 2022).

Heavy metals such as lead (Pb), cadmium (Cd), mercury (Hg) and arsenic (As) have been categorized as having no known functionality or nutritional values in the human body, however, these metals have many industrial uses such as in electroplating, and in making battery components and circuit boards that are used in modern electronics. If found in the human body, they are due to exposure to sources such as ground water, forest fire, volcanic ash, natural erosion, factory, and power plant emissions, regulated release by industry into the natural water ways and also unregulated pollution. Consequentially, these elements are found in detectable quantities in all commercially available food products, including baby foods (Akonnor and Richter, 2021).

The food and drug administration (FDA) takes exposure to toxic elements in food supply very seriously because it stands to protect the health and safety of the youngest and most vulnerable ones in the population. Since most of these toxic elements are present in the environment and cannot be completely avoided, thereby causing them to enter the food chain through soil, water or air, the goal has always been to reduce exposure to these toxic elements in foods to the greatest extent feasible (FDA report, 2021). It has however been reported that there is very little guidance on what levels are nationally and globally considered safe for infants in the 6 to 12 months of age (Akonnor, 2021). As infants transition from breast milk or formula to solid foods, there is limited guidance levels of heavy metals in most of the foods they consume. For example, the guidance level for lead was set at 100 ng/g for candy and 50 ng/g for juice, while the guidance levels for mercury have been limited to fish and fish products only (Akonnor and Richter, 2021).

The consensus recommendation by the WHO, agreed on by other leading baby food industries, is that young infants should drink only breast milk, or if necessary, infant formula until a baby is 6 months old, with water introduced around then and plain cow's milk at around their first birthday. In essence, no juice, flavored or plant-based milks, nor caffeinated beverages or sodas are to be allowed. As such, parents of infants are on the right track as breastfeeding is on the rise (Amira et al., 2018). However, as beneficial as this recommendation is, once children get into the toddler zone, it's a pandemonium, as they are exposed to many unhealthy foods and beverages which are packaged for convenience and are considered promising to make children stronger and smarter. For example, the dietary supplements which is said to boost the immune system, the "squeezy" fruit pouches boasting of three grams of protein and three grams of fiber, the oven-baked stone-ground wheat or "wafflez", the superfood puffs and a baffling array of toddler milks purported to aid brain and eye development are in disguise, foods containing simple sugars, acids, and toxic elements which in many studies are linked to an array of health issues such as poisoning, ill health, autism and some developmental behavioral challenges (Jalili et al., 2015, U.S. FDA, 2017).

Heavy metal food contamination is a global phenomenon posing serious risks on human health and perturbing the ecosystem. Over the years, several studies have raised concerns on the presence of heavy metals in baby foods across the globe (Ogochukwu et., 2019, Parker et al., 2022, Zhang et al., 2020, Afonne et al., 2020 and Munir et al.,2022). However, the report presented by the U.S House of Representatives Subcommittee on Economic and Consumer Policy report in 2021 suggested that baby food manufacturers willfully allowed subpar food testing practices and lenient standards which led to baby food productions with elevated levels of heavy metals such as arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb). Although these heavy metals are found to occur naturally in the environment, it is important to note that they may also be released into the environment as industrial pollutants, finding their way into the food chain via plant uptake, livestock consumption of contaminated water or food and/or agricultural as well as manufacturing processes (Parker et al., 2022). These anthropogenic sources have to be controlled since they lead to increased dietary exposures in children (Bair, 2022).

Excessive exposure to inorganic contaminants such as heavy metals impact all humans, however, infants, toddlers and children are especially vulnerable to their toxicity and carcinogenic as well as non-carcinogenic effects due to their immature development and high food intake versus their body weight ratio. When metal pollutants are accumulated through the food chain, their levels may consequently exceed safe thresholds, leading to toxic damage to physiological functions in living systems (Mejias and Garrido, 2017). Adverse effects of heavy metal contamination in the human body include anemia, developmental and reproductive toxicity, nephrotoxicity, neurotoxic effects and lower intelligence quotient (IQ) (Grandjean et al., 2014, Consumer reports, 2018 and Bair, 2022).

Industrial revolution and economic globalization have greatly contributed to increased diversity of environmental contaminants, including heavy metals. Therefore, several heavy metals and metalloids such as arsenic, lead, cadmium and mercury are commonly detected in food samples and are classified as non-essential to metabolic and biological functions, although they are deleterious in various aspects when their concentrations reach a certain level in living cells (Gall et al., 2015, Rai et al., 2019). Due to emerging issues of food security across the globe, and the inextricable associations linked to human health because of their deleterious effects, the United States Environmental Protection Agency (EPA) and the Agency for Toxic Substances and Disease Registry (ATSDR) have included arsenic (As), lead (Pb), cadmium (Cd) and mercury (Hg) in the top twenty list of dangerous substances (ATSDR, 2007; Xiong et al., 2016a, 2016b; Khalid et al., 2017; Rai, 2018a).

Therefore, the U.S. House of Representatives Subcommittee on Economic and Consumer Policy following the 2021 reports recommended the following: (1) that baby food manufacturers should be required by the Food and Drug Administration (FDA) to not only test their raw ingredients but also their finished baby food products for toxic heavy metals, (2) that manufacturers should be required to report to the FDA toxic levels of heavy metals on food labels, (3) baby food manufacturers should voluntarily remove ingredients and products that are found to have high toxic heavy metals, and (4) the FDA should set clear maximum limits for all heavy metal, especially those with low concentrations such as arsenic, lead, cadmium and mercury which are usually permitted in baby foods because even at very low concentrations, they could cause physiological damages in living system.

Increased transparency on heavy metal content in baby foods will create a balance between producers and consumers for acceptable safe limit levels (Bair, 2022 and fda.gov, 2022).

The monitoring of inorganic contaminants at trace levels for food, environmental and biological purposes are very essential and various analytical techniques such as spectrometry, chromatography and electrochemical methods have been used in this regard (Paiva et al., 2019). The determination of heavy metals in food products, drinking water, surface water, ground water, wastewater has been done using atomic absorption (AAS), inductively coupled plasma optical emission (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS) (Muntean et al., 2013, New Jersey Department of Health 2019, Akonnor and Richter, 2021 and Rubio et al., 2023). All of these analytical methods are approved for metal detection in various samples but the QQQ-ICP-MS is an application method that detects ultra trace contaminants in detecting the smallest nanoparticles of analytes (www.agilent.com). This study has used this highly sensitive QQQ-ICP-MS for the analysis of the metals of interest.

CHAPTER 2

LITERATURE REVIEW

Heavy metal pollution has spread widely across the globe, interfering with the ecosystem, perturbing the environment and causing serious and in most cases, deadly health hazards to humans. The major causes of these issue have been linked to the increased pace of urbanization, changes in land use, and increased industrialization, especially in developing countries of the world, thereby making food security a high priority issue on which sustainable global development quantitatively and qualitatively rests (Parker et al., 2022).

Over the past few years, global concerns have been raised on the adverse effects of unexpected pollutants released naturally and by other means into the ecosystem, resulting in contamination of crops, animals, and humans. The origin of heavy metals in soil can therefore be anthropogenic or natural. The link between anthropogenic sources such as industrial wastes, automobile pollution, agricultural practices and mining activities and soil contamination remains a serious pathway for food chain contamination (Eleboudy, 2017, Rai et al., 2019), creating concerns due to their inextricable association to human health (Toth et al., 2016, Rai et al., 2018, Rai, 2019).

The term “heavy metals” has been widely used in the scientific community, but it lacks a standardized and authoritative definition (Parker et al., 2022). This is because certain metals and metalloids such as arsenic (As), lead (Ph) and cadmium (Cd) are classified as being non-essential to metabolic and biological functions but have been found to be deleterious in various respects (Gall et al., 2015), therefore establishing their need to be included in the top twenty lists of dangerous substances by the United States Environmental Protection Agency and the Agency for Toxic Substances and Disease Registry (ASTDR) (ASTDR,2016, Rai, 2018 and Khalid, 2017). On the other hand, certain heavy metals such as iron (Fe), copper (Cu) and zinc (Zn) are essential components of metabolic processes such as cytochromes and enzymes which are linked to metabolic functioning of biota while nickel is an integral component of urease but can cause human health risks at excessive levels (Marschner, 2012).

Regardless of their essentiality or non-functionality in metabolic processes, higher levels of concentration results in their toxicity in living systems (Munir et al., 2021). For example, increased levels of Iron (Fe), Zinc (Zn), copper (Co) and Nickel (Ni) in vegetables can cause a decrease in percentage biomass and eventually plant death (Mejias and Garrido, 2017). Reports by Akhter, (2004) and Eleboudy et al., (2017) also indicates that higher manganese exposure in children can cause neurotoxic syndrome that affects dopamine balance and behavior control, while excessive accumulation of copper in the brain and cornea may lead to Wilson’s and Menke’s diseases characterized by severe mental retardation and death before three years of age. Also, the presence of toxic heavy metals such as cadmium and lead even in low concentrations in milk have been linked to metabolic disorders, heart failure, cancer and even kidney damage (Licata et al., 2004 and Eleboudy et al., 2017).

Heavy metals are described as metals which have a specific gravity or density of more than 5g/cm^3 in their standard state (Holleman and Willberg, 2001. Eleboudy et al., 2017). Although it is easy to classify heavy metals as elements with cellular toxicity, this term has been extended to include micronutrients that at high concentrations also pose a risk to human health (Mejias and Garrido, 2017). Human exposure to these metals occurs through inhalation, oral ingestion and in a few cases, skin application. A major challenge that ensues from these exposures rests on the balance between the positive effects and side effects of these heavy metals depending on their concentrations in living cells. It is therefore important that their levels be maintained within an appropriate range in order to prevent nutritional deficiencies as well as prevent the health concerns associated with their higher concentrations and bioaccumulation in cells (Munir et al., 2021). Also, the presence of toxic heavy metals such as cadmium and lead even in low concentrations in milk have been linked to metabolic disorders, heart failure, cancer and even kidney damage (Licata et al., 2004 and Eleboudy et al., 2017).

Human exposure to environmental contaminants such as these heavy metals from daily diet is concerning for all age groups, however, children are more vulnerable and susceptible because they consume more food relative to their body weight and developing body systems that are very sensitive to even traceable levels of these elements (Costa et al., 2004, Vogt et al., 2012, Akonnor and Richter, 2021). From six months of age, all children are introduced to complementary food products which provide the necessary nutrients needed for growth and development, and according to the United Nation's child fund (UNICEF), diverse food categories including the consumption of fruits, cereals, vegetables, lean meat and fats, cheese yogurt, salt, and sugar are the alternative sources for effective complementary feeding (WHO, 2020).

Food experts consider cereals, fruits and vegetables as foods that contribute the most dietary exposure to toxic metals in children and their accumulation in the body pose a health risk to them due to their body weight and weaker immune systems (Dominiquez A et al., 2017 and Igweze et al., 2020). Metal toxicity therefore depends on factors such as physicochemical properties, amount ingested and frequency of ingestion, individual health status and their synergistic and antagonistic properties due to the presence of other chemical compounds (Couto de Almeida et al., 2022).

Soil to food crop/vegetable systems is a classic example of abiotic to biotic interactions in the environment which is an indication of food chain contamination (Parker et al., 2022). Soil is the major sustenance of food crops on which humans and animals rely for food and can be perturbed by heavy metals from point sources like energy intensive industries such as coal mines, thermal power plants and chlor-alkali chemical industries such as smelting, electroplating, textiles, leather and e-waste processing, or from non-point sources such as soil/sediment erosion, agricultural runoff, open freight storage, irrigation with wastewater or polluted water, metallo-pesticides or herbicides and phosphate based fertilizer applications, all leading to adverse effects on soil biota (Gall et al., 2015 and Parker et al., 2022). These sources pose a major risk to human health through dietary intake of food crops contaminated by root transfer from soil to plant tissues. For example, many medicinal plants were shown to bio-accumulate various metals such as Cd, As, Cr, Cu and Pb when grown near smelting or industrial areas, while greenhouse vegetables have been found to be highly contaminated with heavy metals such as Zn, Ph and Cd compared to open field vegetables through anthropogenic sources (Bolan et al, 2017). The soil-crop-human/animal heavy metal interaction is shown in Figure 1.

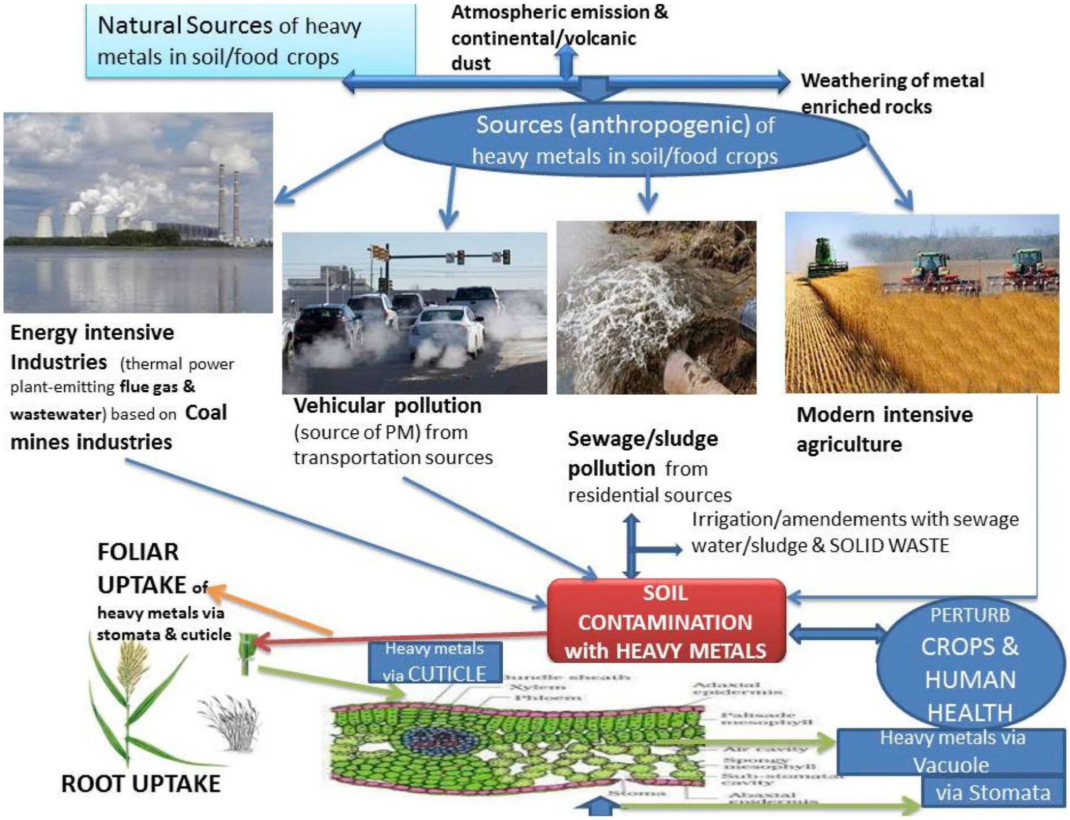

Figure 1: Natural and Anthropogenic Sources of Heavy Metal Contamination in Food Crops and Mechanisms of Their Entrance into Plants Through Stomata/Cuticle with Resulting Effects on Biota and Human Health (Rai et al., 2019).

The rapid pace of industrialization and population growth have created a continuous global scenario for heavy metal contamination leading to bioaccumulation in food crops. In highly populated countries like India and China and underdeveloped countries like Nigeria and Zambia, soil-crop subsystems are usually affected by wastewater irrigation, mining and sludge amendment patterns creating food safety and ecotoxicological consequences. Unlike in Asia and Africa, the sources of heavy metal contamination in America and Europe are found to be particulate matter and modern intensive agricultural practices (Parker et al., 2022). Regardless of the geographical location, food safety is still of major concern due to increasing concentrations of heavy metals and other industrial environmental contaminants (Arisseto-Bragotto et al., 2017).

Infant, Toddlers and Young Children's Exposure to Heavy Metals

After six months of age, baby's nutritional needs drastically shift from active breastfeeding and milk products to food products including mashed fruits, vegetables and cereals which according to the WHO recommendation, are needed for proper growth and development. Unfortunately, the diverse food products introduced as part of complementary feeding exposes children to a high risk of toxicity. A recent report by Ibrahim et al., (2020) showed that among Lebanese mothers, early introduction of infant formula and complementary feedings were common. Unfortunately, the raw materials used to produce baby and young children's foods such as milk, vegetables, fruits and cereals can be contaminated with toxic metals, due to their uptake by plants and animals, although industrial processing and product packaging can also contribute to metal concentration levels when they seep into the packaged food products as a result of the materials used. (Ibrahim et al., 2022 and Lee et al., 2019). A major pathway for heavy metal dietary contaminations in babies and young children is summarized as presented in Figure 2.

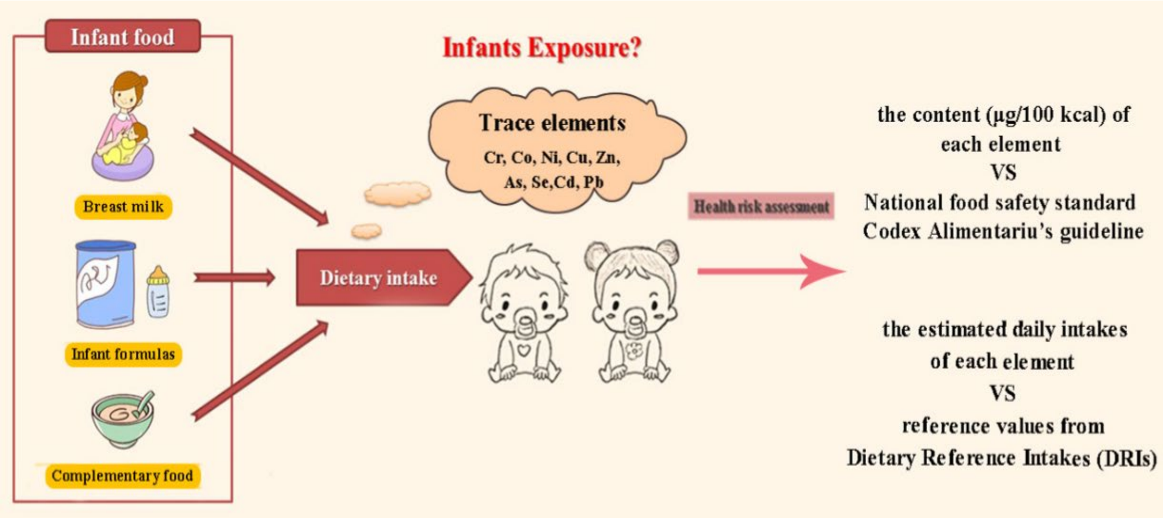

Figure 2: Infant Exposure to Trace Elements from Formula and Complementary Feeding (Lin et al., 2022)

Heavy Metals and Their Mechanisms of Toxicity

Heavy metal toxicity is a problem of increasing significance for nutritional, ecological, and environmental reasons. The most commonly found heavy metals in wastewater are arsenic, chromium, cadmium, lead, nickel and zinc which enter the environment by natural means and through human activities such as erosion, natural weathering of the earth's crust, insect or disease control agents applied to crops and also industrial effluents (Jaishankar et al., 2014). These metals can bind with protein sites and escape control mechanisms such as homeostasis, thereby causing the malfunctioning of cells and ultimately toxicity, with serious adverse effects. A previous study has found that oxidative deterioration of biological macromolecules is due mainly to binding of heavy metals to the DNA and nuclear proteins (Flora et al., 2008 and Jaishankar et al., 2014). Also, heavy metals may act as catalysts for oxidoreductive reactions with oxygen or other endogenous oxidants to produce oxidatively modified proteins and DNA, which has been found to be the possible pathway by which heavy metals induce carcinogenicity (Bair, 2022).

Arsenic

Arsenic is the twentieth most abundant element on earth and it exists in an inorganic form such as arsenite and arsenate compounds which are lethal to the environment and living creatures. It remains one of the heavy metals causing disquiet from individual health and ecological standpoints because it is prominently toxic and carcinogenic due to its availability in the form of oxides or sulfides, or as a salt of iron, sodium, copper and calcium. Humans encounter arsenic by natural means, industrial means or by inhalation. Dietary exposure includes drinking water contaminated with arsenical pesticides, or ingesting food products contaminated with chemicals or mineral deposits. Arsenic is a proto-plastic poison because it affects the sulfhydryl group of cells causing malfunctioning of cell respiration, cell enzymes and mitosis. In cells, inorganic arsenic is bio-transformed through methylation by bacteria, algae, fungi and humans to dimethylarsinic acid (DMA) and monomethylarsonic acid (MMA) as shown in the equation below. MMA (V) and DMA (V) are easily excreted through urine and are bioindicators of chronic arsenic exposure. However, MMA (III) is not excreted and remains inside cells as an intermediate product which is highly toxic and accounts for arsenic induced carcinogenesis (Jaishankar et al., 2014).

iAs (V) → iAs (lll) → MMA (V) → MMA (lll) → DMA (V)

Lead

Lead is a highly toxic metal appearing as a bright silvery metal, and slightly bluish in a dry atmosphere which has no known biological function. It tarnishes on contact with air and forms a complex mixture of compounds (Jaishankar et al., 2014). It is the second metal on ATSDR's list of harmful environmental substances and is very similar to arsenic. Human encounter with lead has been linked with a number of poor health outcomes such as decreased cognitive performance, delayed puberty, stunted post-natal growth and behavioral problems. Early childhood exposure to lead leads to permanent cognitive effects and it has been found in affected individuals in a retrospective study to lead to prolonged developmental delays even into adulthood (Mazundar et al., 2011, Al-Saleh et al., 2017). Lead metal induces toxicity through ionic mechanism and oxidative stress due to the imbalance between the production of free radicals and generation of antioxidants to detoxify the resulting intermediates needed to repair the resulting damage. Sources of exposure to lead include food, smoking, drinking water, industrial processes and domestic sources such as house paints, plumbing pipes, storage batteries, toys and faucets. Since plants take up lead from soil and water, human exposure is mostly through food or drinking water (Jaishankar et al., 2014).

Cadmium

Cadmium is the seventh most toxic heavy metal according to the ASTDR ranking. It is a by-product of zinc production which may be encountered at work or in the environment. Once it is absorbed by humans, it accumulates in the body throughout life. Cadmium is mostly found in fruits and vegetables due to its high rate of soil to plant transfer, causing adverse effects on enzymatic systems of cells where oxidative stress is induced. The mechanism of cadmium toxicity is not fully understood but its adverse effects on cells are known. When bound to certain proteins, the complex created can induce toxicity. For example, when cadmium concentration increases by three thousand folds, it can bind to cysteine rich proteins such as metallothionein which in the liver causes hepatocytotoxicity that can circulate to the kidneys and when accumulated in the renal tissues can cause nephrotoxicity (Jaishankar et al., 2014).

Chromium

Chromium is the seventh most abundant element on earth, occurring in several oxidation states in the environment such as Cr^{2+} to $Cr6^{+}$, although the most common forms are trivalent Cr^{3+} and hexavalent Cr^{6+}, with both states being very toxic to plants, animals and humans. Cr6+ is a stronger oxidizing agent, which when reduced intracellularly can react with biological reductants like thiols and ascorbate to produce reactive oxygen species such as superoxide ion, hydrogen peroxide and hydroxyl radical, leading ultimately to oxidative stress in the cell and causing DNA and protein damage. Naturally, chromium occurs by the burning of oil and coal, oil well drillings and metal plating tanneries. Anthropogenically, it is released into the environment through fertilizers and sewage (Jaishankar, 2014). Cadmium exposure has been linked to increased incidence of attention deficit/Hyperactivity Disorder (ADHD) (Bair, 2022). Cr6+ is categorized as a group 1 human carcinogen by the International Agency for the Research on Cancer because of its mutagenic properties (Jainshankar et al., 2014)

Zinc

Zinc is a heavy metal that is required in small dietary quantities for normal functional metabolism, hence, it is a needed micro-nutrient, being a major co-factor of important proteins and mediating the regulation of several immunomodulatory functions. Its essentiality is directly parallel to its toxicity. It is abundant in the earth's crust and is induced anthropogenically, becoming biologically available through beef and dairy products as well as through medicines in form of supplements (Hussain et al., 2022). Zinc does not typically act as a metal, but when heated at temperatures above 110°C, it becomes malleable, ductile, and reactive with oxygen and other metals. Zinc acts as a cofactor or coenzyme of more than three hundred enzymes and helps to regulate various enzymes, proteins, DNA and DNA binding proteins as well as metabolism. It also helps to suppress the generation of free radicals and reactive oxygen species which underscores protein stability. Zinc deficiency can cause nutritional imbalance, impacting human health and acting as a precursor in the development of certain medical ailments such as immune dysregulation, increased vulnerability to infection, Wilson's disease, and neurological diseases such as schizophrenia, Parkinson's, and Alzheimer's (Agnew et al., 2022).

Zinc consumption is dependent on dietary intake and the average allowance of daily intake according to the Food and Nutrition Board, United States is 11-16 and 7-10g/day for adult males and females. Infants can safely consume 1.4 mg/day. Zinc toxicity in humans is treatable and non-life threatening, however, symptoms such as fever, breathing difficulty, nausea, chest pain and cough are commonly observed. A major challenge with zinc toxicity is the overdosage of zinc supplements which is a major concern for growing infants, toddlers and young children (Hussain et al., 2022).

Aluminum

Aluminum is the third most abundant element in the earth's crust, occurring naturally in the air, water, and soil. Mining and processing of aluminum, however, increases its level in the environment (ATSDR, 2018). Factors that contribute to aluminum toxicity include soil and water pH and organic matter content. With decreasing pH, aluminum toxicity increases, and the mobilization of toxic aluminum starts, which can reduce crop production manifesting as yellowing and death of leaves, chlorosis and foliar necrosis (Gupta et al., 2013). When ingested by humans, aluminum interferes with cellular processes. Although the mechanism of its absorption by the gastrointestinal tract is not fully understood, its toxicity can be detected in seconds or minutes after human exposure. This is because when iron and magnesium are replaced by aluminum, they cause intercellular disturbances associated with communication and cellular growth. These changes are similar to the degenerative lesions observed in Alzheimer patients. However, the greatest complications of aluminum toxicity are neurotoxicity, an example of which is neuronal atrophy (Jaishankar et al., 2014).

Nickel

Nickel is a heavy metal that is found at very low levels in the environment and is found in all soil types, binding with oxygen or sulfur and forming oxides or sulfides in earth crust, with its predominant oxidation state in nature occurring as Ni (II). It is an essential trace element for humans and its deficiency has been linked to reduced iron absorption, anemia and skeletal calcium absorption interruption leading to paraketosis-like damage and altered genetic code transmission as well as growth and reproductive rate impairment. The essential role of nickel, however, consists of the formation of cyclic GMP, which is a signaling agent regulating various physiological processes among which are blood pressure control, sodium metabolism, cardiovascular health and sperm physiology, Excess nickel in cells causes oxidative stress (Das et al., 2017 and Roy, 2020).

Food experts consider cereals, fruits and vegetables as foods that contribute the most dietary exposure to toxic metals in children and their accumulation in the body pose a health risk to them due to their body weight and weaker immune systems (Dominiquez A et al., 2017 and Igweze et al., 2020). Metal toxicity therefore depends on factors such as physicochemical properties, amount ingested and frequency of ingestion, individual health status and their synergistic and antagonistic properties due to the presence of other chemical compounds (Couto de Almeida et al., 2022).

A very major concern with heavy metal toxicity is not locked only in its bio-availability in the environment but rather, it is embedded in the environmental influences such as acidity, alkalinity, adsorption, pH, temperature and speciation, which informs their eventual solubility, availability, mobility and accessibility, hence redefining their chemistry of bio-accumulation and bio-augmentation in the food chain and eventual adverse health impacts when ingested (Nkwunonwo et al., 2021). The overall summary of heavy metal toxicity is summarized in Figure 3.

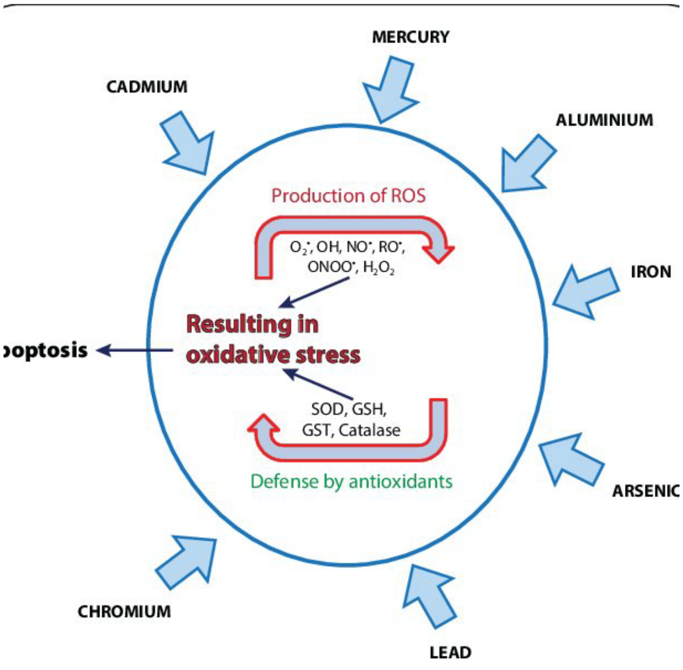

Figure 3: Heavy Metal Toxicity in Cells and the Balance Between ROS Production and Antioxidant Defense (Parker et al., 2022)

Another major aspect of metal toxicity is the exploration by scientists of the link between heavy metal intoxication and autism spectrum disorder (ASD) as well as attention deficit disorder also known as ADHD in children. Since the etiology of ASD remains largely unknown, possible causes are linked to genetic, epigenetic and environmental factors such as ingestion of foods contaminated with heavy metals either maternally prior to pregnancy or direct ingestion through baby foods (Gamakaranage, 2016). Hence, there is conflicting evidence to this effect. Blood, urine and hair samples of autistic individuals were evaluated and some reports found significantly higher levels of all observed metals in studied autistics compared to controls, with positive correlations between evaluated risk factors for heavy metal exposure such as fish consumption, smoking and use of aluminum pots, although there were no significant correlations however, between the recorded levels and severity of autistic manifestations (El Safety, 2012, Gamakaranage, 2016, Zaky, 2017). Other reports however, revealed no significant association between heavy metals and these neuro-developmental disorders (Gil-Hernandez et al., 2020, Jafari et al., 2020 and Nayak et al., 2021).

To ensure safety, the U.S Food and Drug Administration regulates the metal content of food products. Additionally, non-governmental organizations such as the Consumer Reports and Healthy Babies Bright Futures (HBBF), especially since 2017 have been more committed to ensuring that food products, especially baby, toddler and adolescent food products are tested for the presence of heavy metals (Parker et al., 2022). In 2021, the US FDA issued the Closer to Zero (C2Z) action plan for foods eaten by babies and young children to monitor and reduce toxic element exposure to as low as possible through food (Flannery et al., 2022). The C2Z's goal is to continuously monitor dietary exposure to heavy metals, keep the concentration levels as low as possible, prioritize foods commonly eaten by babies and young children due to their extreme vulnerability to the harmful effects of these metals while still maintaining access to nutritious foods. The overall plan is depicted as seen in figure 4 below (FDA.gov).

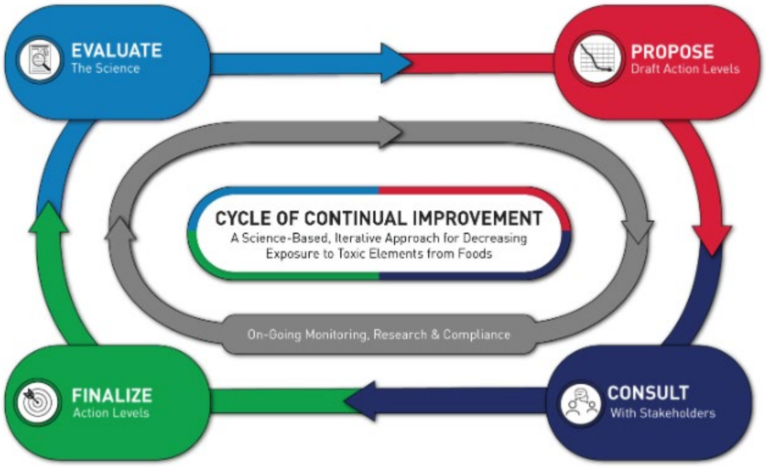

Figure 4: FDA's Closer to Zero Plan Approach Uses a Science-based, Iterative Approach for Achieving Continual Improvements Over Time

Although so much work has been done globally on heavy metal toxicity, it is alarming to know that daily threshold limits are the sole responsibility of various regulatory agencies for metals such as aluminum, arsenic, and mercury while cadmium has no set guidance level in various baby foods. (Igweze et al., 2020). Despite the reports by various studies linking chronic aluminum to Alzheimer's disease as well as neurologic, skeletal, and hematopoietic health issues in individuals with impaired renal function, as a result of arsenic use in agricultural planting processes, the use of rice in infant formulas has received little or no attention (Igweze et al., 2020 and Bashiry et al., 2021).

One major way that addresses the subpar testing practices and lenient standards employed by different baby food manufacturing in regulating heavy metals in baby foods will be to recommend for testing products, a single sample preparation method that could be effective in analyzing the largest number of quantifiable elements with instruments that will yield the highest recovery that is above 95%. A study by Yang et al., (2017) clearly demonstrated the high temperature and high-pressure single reaction chamber (SRC) microwave digestion as the best microwave-assisted acid digestion procedure that yielded average recoveries ranging from 93% to 113%. The study also showed that of all the inductively coupled plasma optical emission spectrometers, the QQQ-ICP-MS (Model 8800) method of metal analysis is the most efficient method for determining low abundant trace and ultra trace element. Based on this finding, the SRC- microwave digestion and QQQ-ICP-MS has been used in this study.

Due to the many conflicting theories on the mechanisms of disease and disorder developments in children that are linked to different aspects of heavy metal consumption, metabolism, bioaccumulation, and regulation in food products, there is an urgent need for large-scale studies that can clearly establish the absolute mechanisms by which metal toxicity affects the general well-being of children and possible ways to alleviate the effects that have been already evaluated.

The main aim of this study is to evaluate and assess heavy metal concentrations in selected baby and toddler foods in the Houston area. Specific objectives are:

1. To assess heavy metal levels in selected food samples and determine if the current safe limit levels are exceeded.
2. To assess the concentrations of these heavy metals and determine if organic baby products are safer than natural baby products

3. To determine the likely sources of heavy metal contaminations in the evaluated food products based on what food type has the most heavy metal levels
4. To predict the effectiveness of the FDA’s C2Z plan based on heavy metal levels in selected food products

CHAPTER 3

DESIGN OF THE STUDY

Collection of Samples

For this study, the inclusion criteria included foods from birth through complementary feedings to toddler age groups which were targeted toward children between the ages of zero to five years (infancy to toddlers), and that contained one or more primary ingredients in the following categories: dairy, fruits, vegetables, root vegetables, grains, sugar supplement and juice; and are always accessible and most commonly purchased off of the shelf brands as opposed to homemade foods. Three samples of each type of ten different food products were purchased from Walmart local supermarket in Houston, Texas (making a total of 30 in all). These food types represent baby food products produced by the top leading brands of baby foods that could be purchased from many stores across the Houston area and anywhere in the United States.

These included two different brands of infant dry milk formulas, two brand of stages two and three baby food puree labeled “Organic”, representing a brand that creates their products with ingredients grown using organic farming practices, while the remaining six brands were labeled “Natural”, representing brands that make their products with ingredients produced using standard farming practices. All products were packaged in either plastic tubs, glass jars or pouches and cans. The products were either a single type of fruit or vegetable or a mixture of two or three items from the category listed above. All samples were purchased safely for consumption and analyzed in triplicates to capture the potential variability in heavy metal concentrations with each product type. Table 1 provides the details on each sample including the ingredient categorization, brand, organic designation, and packaging material.

Table 1: Food Sample Types and Consumer Age Group Category

Sample ID	**Primary ingredient(s)**	**Food category**	**Packaging material**	**Size**	**Consumer age group**
S1 **Brand1**	Sweet potato and turkey puree	Natural	Squeezable pouch	3.5 oz	Stage 3: 6-12 months
S2 **Brand 2**	Banana and raspberries fruits	Natural	Squeezable pouch	3.5 oz	Stage 2: 6-9 months
S3 **Brand 3**	Sweet potato and apple puree	Organic Non-GMO	Squeezable pouch	4 oz	Stage 3: 6-12 months
S4 **Brand 4**	Caprisun	Organic Non-GMO	Squeezable pouch	4.5 oz	Stage 3: 6-12 months
S5 **Brand 3**	Chicken casserole with vegetables and rice puree	Natural	Plastic	16 oz	Stage 2 supported sitter 4-6 months
S6 **Brand 5**	Infant formula A	Natural	White vanish lined Aluminum can	12.4 oz	Toddlers 2-6 + years
S7 **Brand 5**	Rice cereal	Natural	Plastic	2.5 oz	Sitter: 6-8 months
S8 **Brand 6**	Infant formula B	Natural	White vanish lined Aluminum can	12.7 oz	Toddlers 2-6 + years
S9 **Brand7**	Syrup	Natural Non-GMO	Plastic	6fl/ oz	Stage 1: 0-12 months
S10 **Brand 5**	Beef and Gravy	Natural Non-GMO	Glass jar	4 oz	Stage 1: 0-12 months

Sample and Standards Preparation

After purchase, all the ten baby food samples purchased in triplicates, were stored and sent in their original, sealed packaging to the ICP Laboratory in the Center for Advanced Analytical Geochemistry (CAAG) at the University of Houston, Texas. Food samples were analyzed in triplicates using the Single Reaction Chamber (SRC) Microwave digestion method. This system operates at a maximum power of 1500W (Table 2). For each food sample,500 mg as loaded into 40 ml quartz tubes and 5 ml of 16 N HNO_3 with 1 ml of 12 N HCl and 1 ml of 30% H_2O_2 were added into each tube. The tubes were loaded into the SRC microwave system for digestion. After digestion, the solutions were transferred and comingled into a single PFA beaker, then dried down incipiently on the hot plate at about 180ºC. Afterwards, 2% HNO_3 was added into the PFA beaker and heated for 30–90 minutes at about 180°C to assure re-dissolve. The sample solution was then diluted to about 4-g solution at a low dilution factor (≈10x) to form the final solution for determination of low abundance trace and ultra-trace elements. Samples were then analyzed for total Al, Cr, As, Cd, Zn, and Pb with the exception of Hg using the QQQ-ICP-MS (Model 8800) method according to Milestone's UltraWAVE method (Technical SOP for Operation of Milestone UltraWAVE Microwave Digestion Unit). The parameters for the QQQ-ICP-MS employed in this study are as presented in Table 2.

Table 2: Operating Conditions of the QQQ-ICP-MS/UltraWAVE SRC System

Parameter	**Setting**
RF Power	1500W
Temperature range	90ºC min-300ºC max
Pressure range	15 MPa to 19.9MPa
PTFE liner material	Pure Nitrogen (N_2)
Nebulizer	Concentric
Plasma gas flow rate (L/min)	1.8
Carrier gas (L/min)	1.07

Statistical Analysis:

Values are expressed as mean ± SEM of triplicate measurement. Analysis of variance was performed to compare the differences among the groups using (SAS, 2016 version 9.4). Linear regression analysis was also performed to statistically link the concentrations of the heavy metals with the packaging materials used for the different food products. A P value greater than 0.05 was considered insignificant.

CHAPTER 4

RESULTS AND DISCUSSION

Table 3 shows the mean value concentrations of the heavy metal contents. There were 10 food types used.

Table 3: Mean Value Concentrations of the Heavy Metal Contents of the 10 Food Types

Food type	(Al) µg/g mean (SD)	(Cr) µg/g mean (SD)	(Ni) µg/g mean (SD)	(Zn) µg/g mean (SD)	(As) µg/g mean (SD)	(Cd) µg/g mean (SD)	(Pb) µg/g mean (SD)
S1	0.217 (0.060)	0.045 (0.012)	0.220 (0.202)	1.617 (0.014)	0.011 (0.001)	0.003 (0.000)	0.004 (0.001)
S2	4.089 (0.172)	0.136 (0.098)	0.414 (0.300)	1.346 (0.024)	0.012 (0.001)	0.001 (0.000)	0.001 (0.000)
S3	2.501 (0.355)	0.267 (0.046)	0.148 (0.004)	1.304 (0.092)	0.013 (0.001)	0.002 (0.001)	0.003 (0.000)
S4	0.316 (0.048)	0.013 (0.003)	0.019 (0.004)	0.139 (0.012)	0.006 (0.001)	0.000 (0.000)	0.000 (0.000)
S5	0.824 (0.060)	0.048 (0.005)	0.065 (0.006)	2.90 (0.0059)	0.013 (0.001)	0.004 (0.001	0.001 (0.000)
S6	0.353 (0.000)	0.032 (0.001)	0.095 (0.001)	33.553 (1.5689)	0.023 (0.001)	0.000 (0.000)	0.002 (0.000)
S7	0.492019 (0.0375)	0.067 (0.008)	0.235 (0.011)	69.535 (3.198)	0.102 (0.004)	0.017 (0.001)	0.001 (0.000)
S8	0.416 (0.034)	0.037 (0.013)	0.069 (0.016)	30.247 (1.497)	0.012 (0.000)	0.000 (0.000)	0.000717 (3.457)
S9	0.085 (0.023)	0.010 (0.003)	0.015 (0.002)	0.063 (0.020)	0.008 (0.001)	0.000 (0.000)	0.000 (0.000)
S10	0.271 (0.023)	0.023 (0.008)	0.020 (0.001)	23.694 (1.346)	0.009 (0.001)	0.000 (0.000)	0.001 (0.000)

- SD refers to the standard deviation.
- µg/g =mg/kg=ppm

The metal contents of baby foods are continuously gaining attention and importance because of their toxicological and nutritional viewpoints. The United States FDA has listed dietary intake as the major supplier of most toxic metals to the pediatric and human population generally. Therefore, the need to investigate the levels of toxic metals in commonly purchased baby food products, especially for the recently reported heavy metals demand attention. Different food products have been analyzed for different elements up to ug/kg levels using different techniques such as AAS, ICP-OES, ICP-MS and QQQ-ICP-MS. Owing to the peculiar characteristics of ICP-MS having low detection limits, multi-elemental capacity and wide linear range of measurement, there has been an increasing number of scientific papers reporting the analysis of food samples by ICP-MS over the years (Sahan et al., 2007). The QQQ-ICP-MS however, presents higher detection limit levels and greater percentage recoveries and has been the method of choice for elemental analysis in our study. The findings from this research presents the different evaluated metals.

Aluminum

In this study, aluminum was detected in all the food samples at varying concentrations as seen in Figure 5 below. However, the highest levels were observed in food types S1 (Pear, banana and raspberry fruities), S3 (sweet potato and apple puree) which is organic, S5 (rice puree and chicken casserole) at concentrations of 4.09 µg/g and 2.50 µg/g These three food types exceeded the proposed minimal risk level of 1 ug/g. Aluminum is considered ubiquitous because it is the third most abundant element in the earth's crust, being unavoidably present in tap water, evaporated salt, white sugar, and in most ingredients, such as flour, and baking powder used in making cookies for our children. There is no evidence from previous studies that children are more sensitive to aluminum than adults, but its toxicity has been linked to kidney problems when its concentration exceeds the minimal risk level of 1 ug/g over time (Chuchu et al., 2013). The lowest level of Al was observed in S9 (syrup) at a concentration of 0.09 ug/g. Syrups are made of sugar and water and the brand evaluated in this study listed corn syrup and high fructose corn syrup as the main ingredients, it also contained less than two percent of natural and artificial flavors and preservatives, which could be the reason it is safely below the minimal risk level for the Al. The information is presented in Figure 5 below.

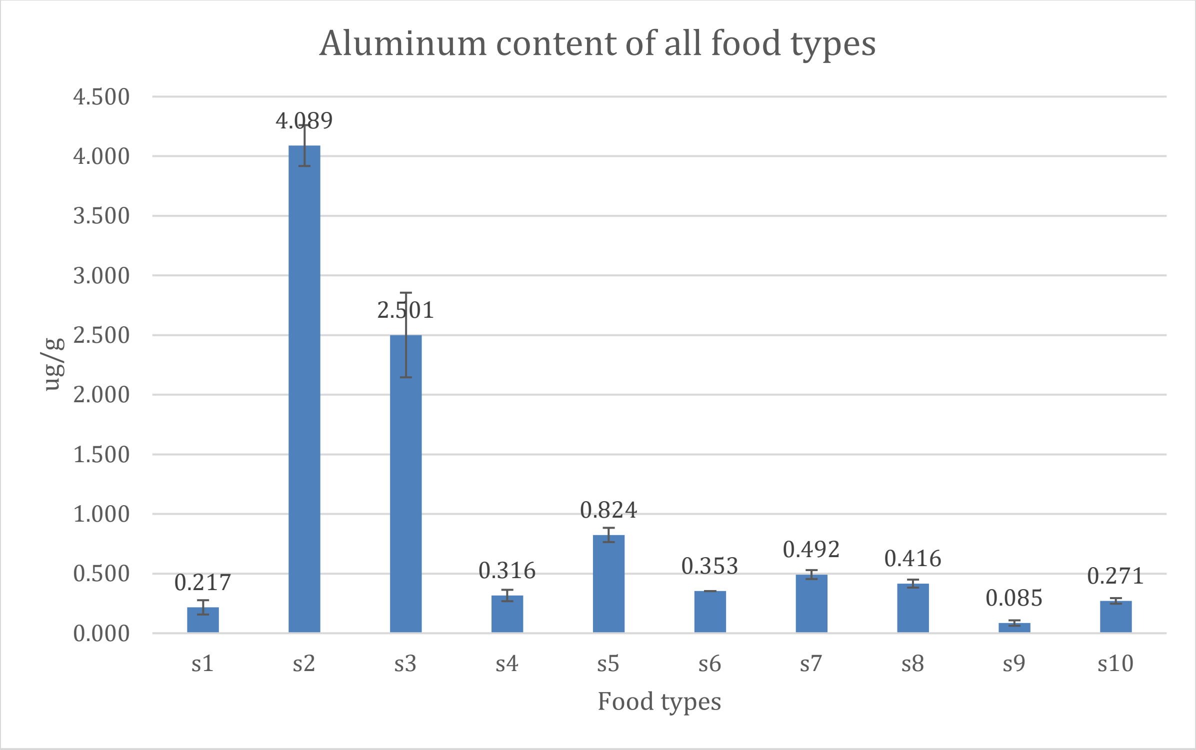

Figure 5: Aluminum Content of all Food Types

Chromium

Chromium was also detected in all the evaluated food types, although the highest levels were recorded in S2 at 0.13 ug/g (pear, banana and raspberry fruities) and S3 at 0.27 ug/g (sweet potato and apple puree) which is an organic food product as seen in Figure 6 below. The minimal proposed limit level for chromium is 0.1 ug/g and these two-plant product formulated food samples exceeded this limit. Chromium has been described to be an essential trace element that can improve insulin sensitivity and enhance metabolism. Its deficiency includes weight loss, reduced response to glucose in the blood, which increases the risk of diabetes along with many other factors in the body. Its abundance is usually observed in sweet potatoes and broccoli (Antoniouss et al., 2011) agreeing with the finding from this study. The lowest levels were observed in S9 (syrup) and S4 (Caprisun) which are mostly non-plant-based sugar products, serving children in the 1–2-year age groups. Caprisun's main ingredients are water, sugar, and some fruit concentrates. Chromium levels are produced in Figure 6.

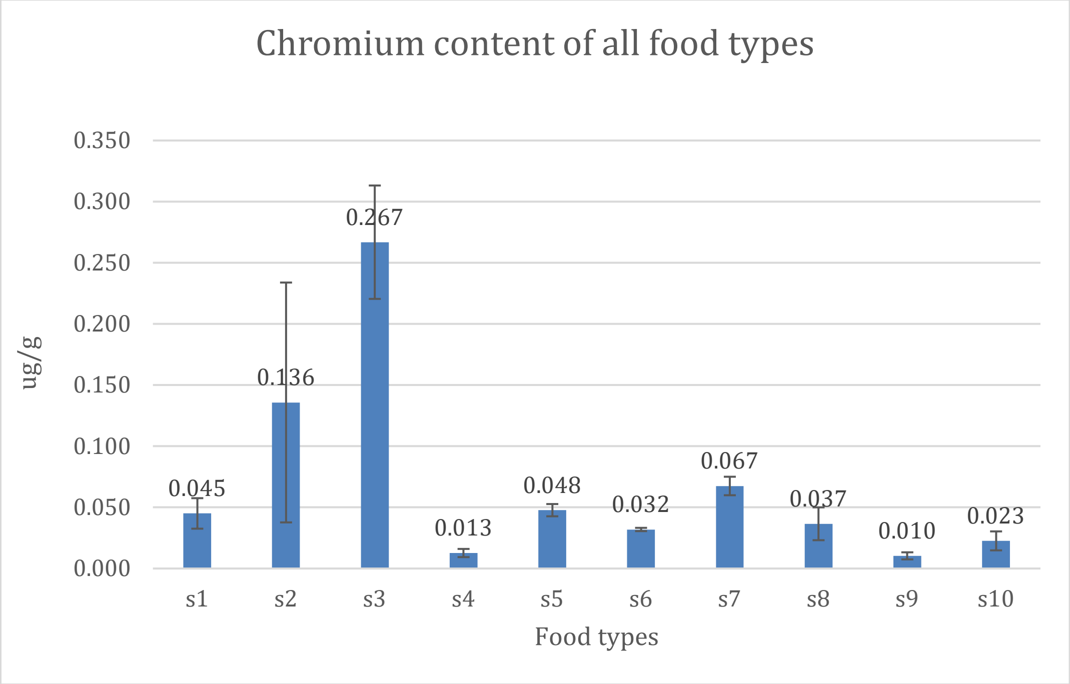

Figure 6: Chromium Content of All Food Types

Nickel

Nickel levels were highest in food types S1 (Sweet potato and turkey puree), S2 (banana and raspberries fruities, S3 (organic sweet potato and apple puree) and S7 (rice cereal). The concentration levels were 0.2 ug/g, 0/4 ug/g, 0.14 ug/g and 0.22 ug/g respectively (Figure 7). Nickel levels are usually low in the environment, and they exist in the earth's crust, however, the use of nickel in modern technology is gradually increasing their widespread release and accumulation in different locations of the earth. (Roy 2020) It is an essential trace element needed for metabolic processes. Its deficiency affects calcium absorption into skeleton, leading to paraketosis like damage and has also been linked to reduced iron reabsorption which could lead to anemia. Being an essential element, its toxicity due to increased concentrations in the body increases DNA methylation and gene expression inactivation (U.S. Department of Health report, 2005)). The lowest level of nickel was observed in S9 (syrup) at a concentration of 0.01 ug/g.

The ASTDR minimal risk level for nickel is only available for inhaled nickel, no oral standard is set for nickel, and it is mostly ignored as a heavy metal. There is need for the FDA to set a clear minimal oral risk level standard for nickel due its accumulative chronic potentials (www.atsdr.gov).

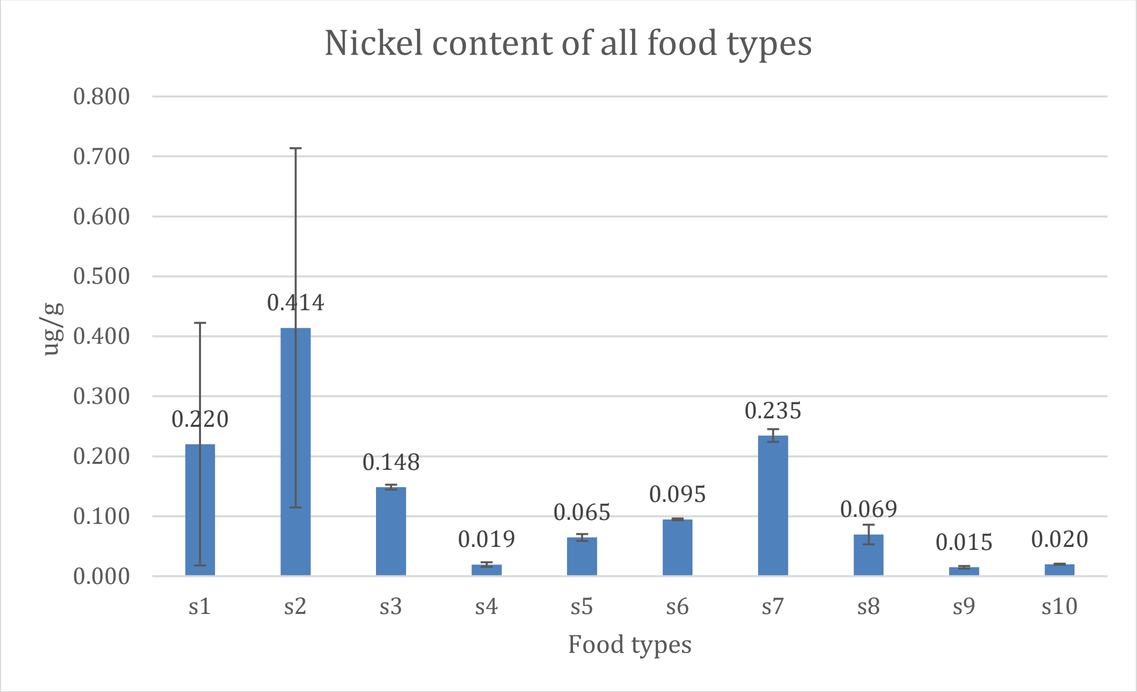

Figure 7: Nickel Content of All Food Types

Zinc

Zinc was the most elevated metal overall in this study (Figure 8). The highest levels were observed in the two brands of infant formula (S6 and S7), rice cereal product (S7) and beef and gravy (S10). Zinc is a ubiquitous element that is abundant in the earth's crust, water, food additives, fruits, and vegetables. It is an essential trace element and an important co-factor for many proteins in the human body. It is essentially important during fetal growth and development and is usually used to fortify various products such as infant formula and as medicinal supplements when needed (Hussain et al., 2022). Zinc does not typically act as a metal because it lacks an 8-electron octet, however it forms a stable metal ion in biological matrices where the redox potential is in constant flux. The ASTDR minimal risk level for zinc in infants and toddlers is 0.3 ug/g/day. In this study, the MRL was greatly exceeded in all four that exhibited the highest levels as seen above. This could be as a result of zinc bioavailability linked to dairy products, beef and nuts. Because of its persistence in the human body as well as in the environment, Zinc toxicity is linked to its overdosage, although its symptoms are treatable and non-life threatening. Symptoms linked to overdosage of zinc are (but not limited to) fever, dizziness, breathing difficulty, chest pain nausea and cough (Hussain et al., 2022). The zinc levels observed in this study are presented in Figure 8.

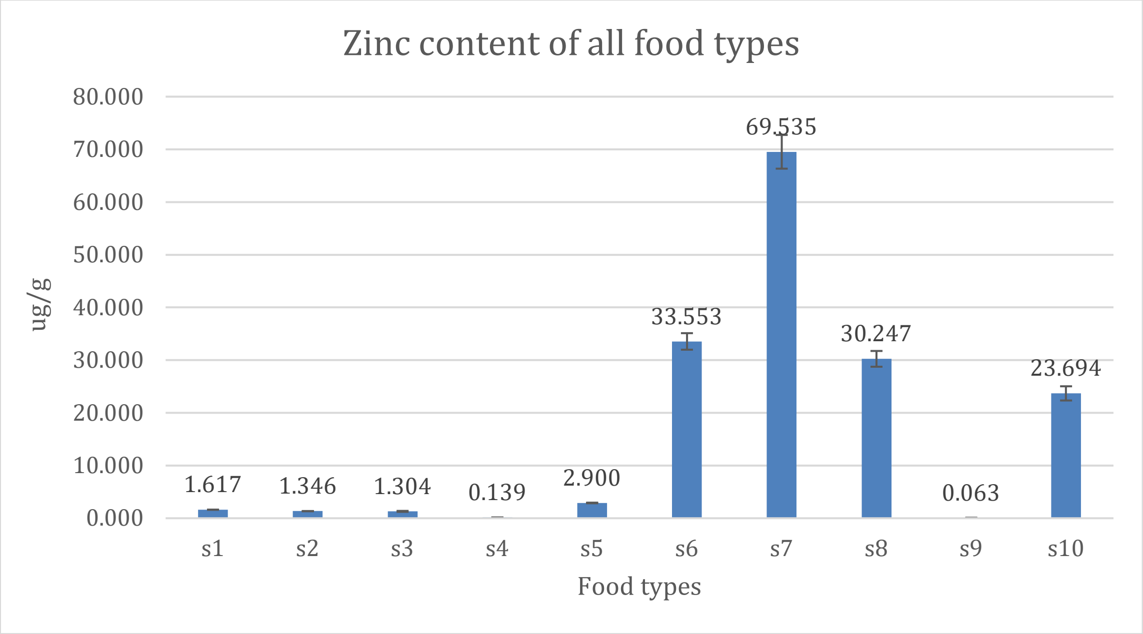

Figure 8: Zinc Content of All Food Types

Arsenic

Arsenic levels were elevated the most in two food types, S6 (Infant formula A) and S7 (rice cereal) at 0.02 ug/g and 0.1 ug/g although all other food types had lower concentrations which did not exceed the minimal risk level of 0.01 ug/g while Caprisun had the lowest concentration of 0.006ug/g as seen in Figure 9 below. Arsenic is a component of the earth's crust and groundwater and most data in literature reported the evaluation and measurement of total arsenic (Hojsak et al., 2015). Quantification of inorganic arsenic is difficult and efficient methods have only recently been available although still not widely used in studies like this. Rice and rice products have been shown to have high arsenic levels because it is a semi aquatic plant and the abundance of arsenic in groundwater will be a factor for its elevated levels in this food type Inorganic arsenic is largely responsible for its toxicity and has been considered to be a first level carcinogen because chronic arsenic contamination due to long term exposure can lead to skin cancer, skin pigmentation and hyperkeratosis (Hojsak et al., 2015). In greatly exposed children, inorganic arsenic has been reported to be related to the development of cancers and lung disease later in life (Liaw et al., 2008).

In this study, arsenic was elevated although slightly in one of the infant formula brands. Studies by Signes-Pastor et al., 2017 and Pizalli, 2019 had indicated the presence of arsenic in infant formula and arsenic exposure to humans have been shown by studies such as Jackson et al., 2012 to be pervasive and its effects seen even at low concentrations. Drinking water is a main route of exposure for many individuals, including babies and young children. Globally, arsenic content in rice should be continuously monitored and kept to as low as possible in infant and toddler food products and in cases where they are found in infant formula, their presence could be due to the water source which could contribute to the slightly elevated value observed in this formula. The result of arsenic concentrations is as presented in Figure 9.

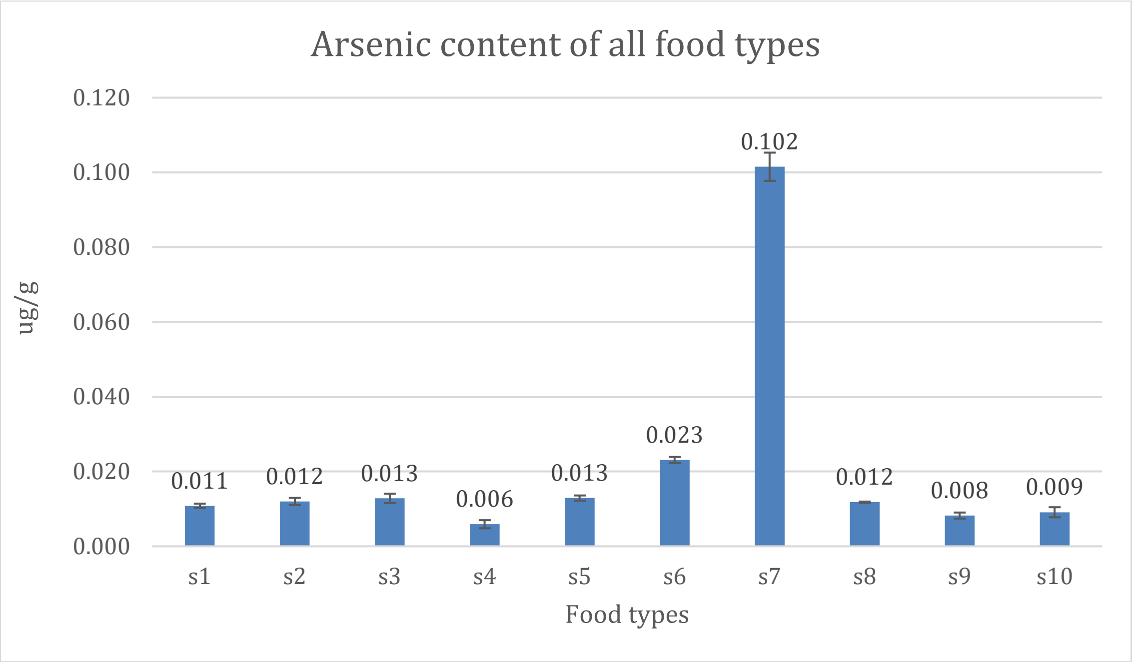

Figure 9: Arsenic Content of All Food Types

Cadmium

Cadmium levels in this study were generally low and closer to zero as seen in Figure 10 below. The only food type that had the highest leel tthat exceeded the minmal risk level of 0.005 ug/g was S7 which is a rice rpoduct whille the lowest level was observed in S4 (Caprisun) at a concentration of 0.00007 ug/g. Rice and cereals are a major source of dietary toxic metal such as cadmium. The observed concentration in rice cereal in this study was 0.017 ug/g. Cadmium is found in the earth's crust and the primary source of human exposure is a consequnce of food chain contamination. It is a by product of zing mining, and is a metal that competes with essential elements like zinc, copper or iron, interfering with metabolic pathways in the body. (Rubio et al., 2023). Its toxciity can chronically induce renal damage, bone softening and cardiovasculr diseses. A commonn example of cadmium posoning is the itai itai diseases whcuh was observed in Japan in the 1970s as reported by Aoshima (2021) . The polluted irrigation water used for drinking by residents close to the Jinzu river where zinc was mined developed cadmium poisoning. According to the ASTDR, cadmium is found mainly in cereal and cereal products and this agrees with the finding from this study. The lowest level was also observed in Caprisun.

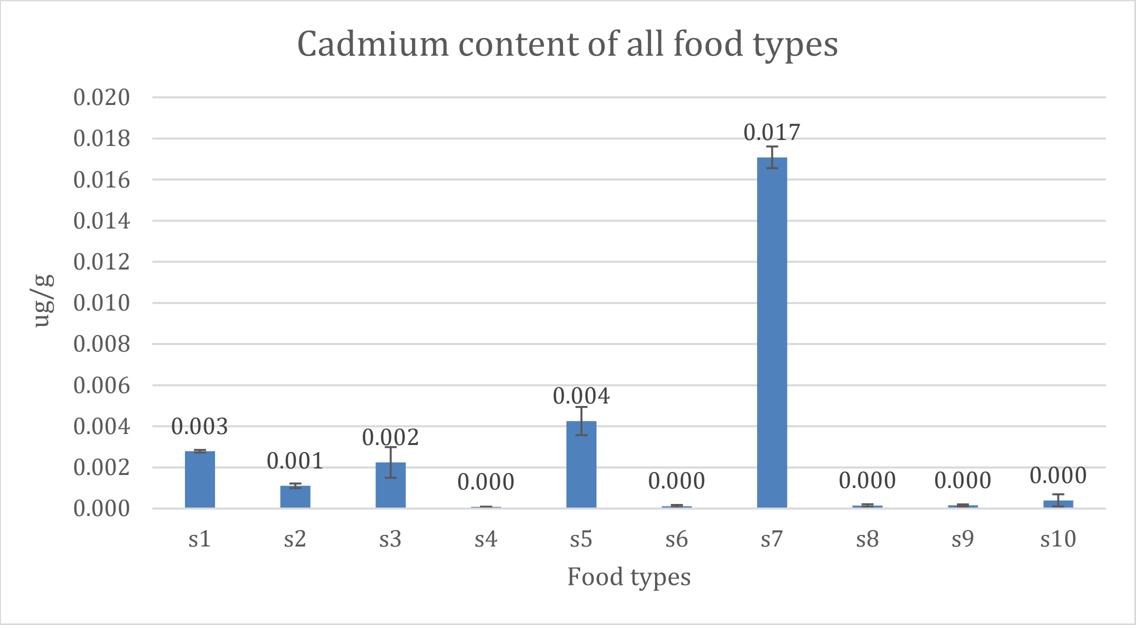

Figure 10: Cadmium Content of All Food Types

Lead

Lead is naturally present in the earth's crust and has been listed by the WHO as a major health concern. In this study, lead levels were generally low and although they were detected in all the food samples, none of them exceeded the minimal risk level of 0.005ug/g as seen in Figure 7. Lead toxicity is attributed to its affinity for thiol groups and its ability to substitute calcium and zinc causing lead poisoning which can cause health problems in children. Cereal based products have been shown in previous studies to contribute the most lead to infants and young children as reported by Muntean et al., 2013, however, none of the results obtained in this study exceeds the minimal risk level.

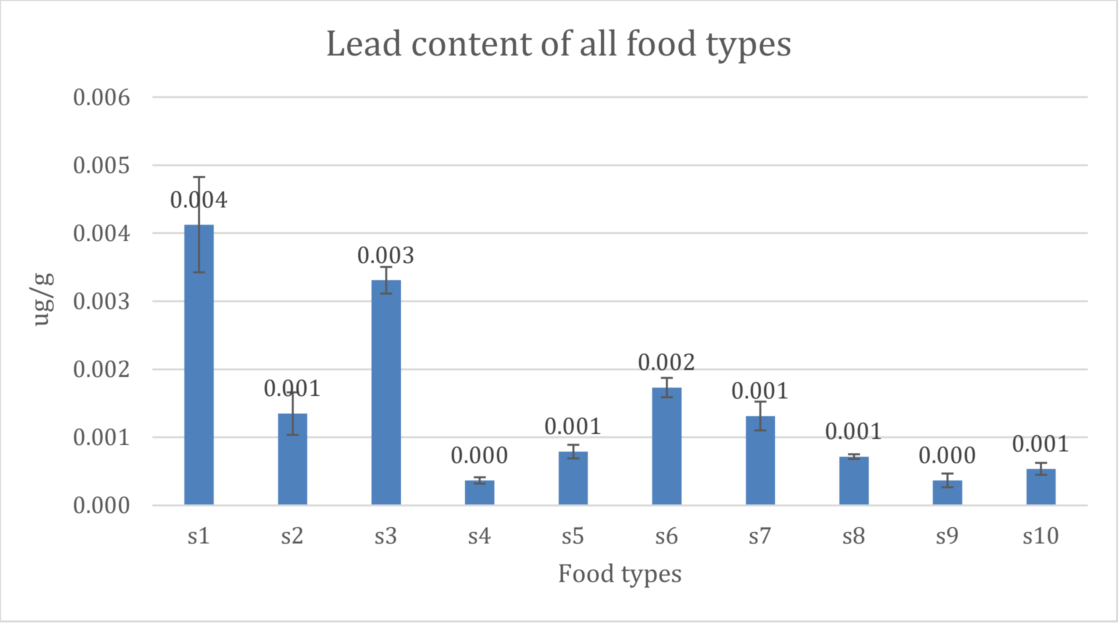

Figure 11: Lead Content of All Food Types

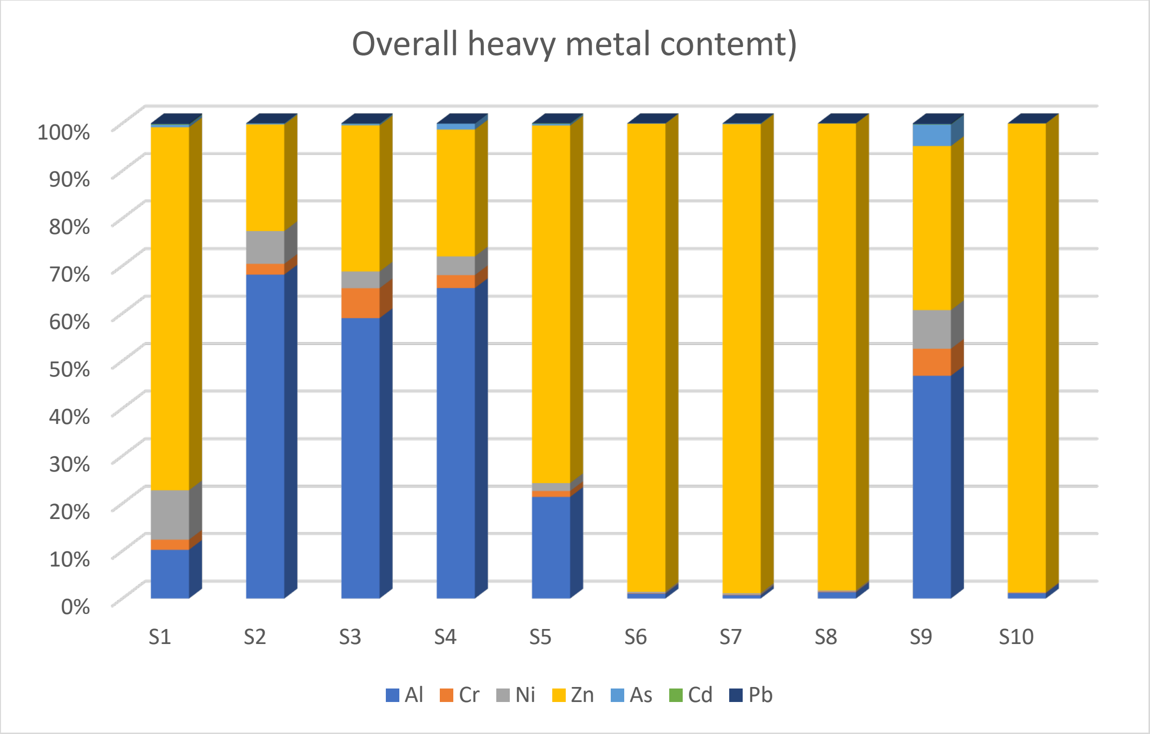

Figure 12: Comparative Description of the Overall Metal Content Expressed in Percentages

In this study, zinc and aluminum were the most elevated metals overall, while lead and cadmium were the least observed. Arsenic and chromium were also low but were detected in food types such as rice and sweet potato which have been reported in various studies as being major sources of dietary exposure to indicated metals. Baby food products formulated directly from plant products, grown and cultivated as being either organic or natural were the main sources of the most metal levels in this study. This suggests that food to crop system contamination is still a major source of toxic metals in most baby food products as well as a possible water source contamination. The likely ways by which this food chain contamination affects food products could stem from the FDA' s report which indicated that baby food manufacturers were mostly concerned with testing raw ingredients and not finished products. If raw ingredients are tested, there is need to also test the finished products for heavy metals as water remains one of the indices for toxic metal persistence in the environment. Ensuring that standard testing is done before and after food production will guide the regulating agencies in determining the possible sources of toxic metals in baby foods.

Accurate dietary assessment is a critical aspect for monitoring toxic elements in food and this depends on human exposure and the risks associated with its cumulative effect over time. Risk assessment studies determine the content of each element in various food products and evaluates the standard guidelines that determines a safe limit level of these elements based on daily intake or concentration of each element in the food type. (Kordas er al., 2022). Therefore, the recommended daily dietary allowance of heavy metals is dependent on several factors which include but are not limited to the estimate of the daily human exposure to substances that are hazardous, food type, bioaccumulation of such substances, body weight, and risk assessments over a duration of exposure. Several terms such as oral reference limit, tolerable upper intake level, or minimal risk levels are used by different agencies to describe these assessments. As a result of the factors considered in setting intake limits, the values set by different agencies sometimes differ from each other. Regardless of how these terms are evaluated, the main objective of agencies working to provide these limits is to monitor and control human exposure. Therefore, in this study, we used the minimal risk level values (MRLs) of the Agency for Toxic Substances and Disease Registry (ATSDR) which is developed jointly with the U.S. Environmental Protection

Agency (EPA) and is based on the toxicological profiles of the metals of interest (Minimal Risk Levels for Hazardous Substances | ATSDR (cdc.gov)

In this study, seven heavy metals of interest in seven different leading brands of ten easily purchased off-of-the-shelf infant and toddler foods in the Houston area of the United States were evaluated, and it was observed that aluminum and zinc were the two most elevated metals in all the food types as seen in Figures 12. The highest concentrations of aluminum were observed in food samples S2 and S3, (4.09 µg/g and 2.50 µg/g) which are stages 2 and 3 food categories, providing nourishment for infants within the 6-9 months age group (Figure 12). Descriptively, these two food types are purely formulated from plant products which could explain why the elevated concentrations of Al in these two food samples. This could be a result of the food chain contamination during the planting process since aluminum is very abundant in the earth's crust. Food samples S2 and S3 greatly exceed the oral MRL of aluminum which is set at 1 µg/g a day. Although both food products were packaged in squeezable pouches of similar packaging material and produced by different manufacturers, no significant differences were found in the metal concentrations based on the packaging material used (P= 0.63, std error 1.94). A previous study by Chuchu et al., (2013) observed elevated levels of aluminum in infant formulas, attributing this to the soy content of dairy products. Our study, however, observed lower levels in similar food products at levels below the MRL as seen in Figure 12.

Zinc is the second observed most elevated metal in our study. It is ubiquitous and very essential for life, helping to maintain and balance homeostasis. Hence, it is referred to as an essential macronutrient and as such can be harmful if deficiency sets in. The ATSDR

MRL of zinc for infants and toddlers is 0.3 µg/g daily while the WHO/FAO sets the upper limit intake at 7 µg/g /day (Wolf and Rink, 2022). Our result indicates a highly-elevated concentration of zinc in food samples S6 (an infant formula) - 33.5 µg/g, S7 (rice cereal)-69.5 µg/g, and S8 (an infant formula) -30.2 µg/g. as seen in Table 3 and Figure 8. Zinc is distributed in a wide range of food including meat and meat products, grains, cereals, dairy products, and dietary supplements (Li et al., 2022). This could explain the reason for the elevated concentrations in the food samples which are infant formulas of different brands and rice cereal food products respectively. Although zinc plays an important role in multiple metabolic processes such as protein synthesis, immunity, and gene expression, having over a thousand-transcription factors regulating over 300 enzymes in the body, excessive consumption could exert immunosuppressive effects that could promote pathogen multiplication, diarrhea, stomach pain, nausea, and vomiting, all of which are non-life threatening and are treatable (Li et al., 2022).

In Figure 7, nickel levels were highest in food products that were plant-based ranging from rice cereal (S7) to sweet potato and apple mix (S3) to pear, banana, and raspberries (S2) and sweet potato and turkey puree (S1). The concentration values were between 0.14 µg/g -0.41 µg/g which did not exceed the proposed MRL. Nickel is also an essential trace element whose biological functional importance has not been clearly demonstrated. Its deficiency however is linked with effects such as abnormal cellular morphology, oxidative metabolism and lipid level fluctuations in the body. Since little is known about the essentiality of nickel in humans, nickel dietary recommendations have not been established for humans, creating the need for more studies, cellular or biological studies that can attend to this ignored element (www.atsdr.cdc.gov).

Cadmium and Lead levels overall were generally low and none of the food types evaluated exceeded the recommended permissible limit as seen in Table 2. The highest concentrations of lead were observed in S1 and S3, (Figures 10 and 11) both of which contained sweet potato in their list of ingredients. This finding is consistent with Parker et al., (2022), and Gu et al., (2020). Since lead is found occurring in small quantities in the earth's crust, this could explain why both food products which are plant-based, recorded the highest concentrations as observed overall in the study although it did not exceed the MRL of 0.005ug/g.. Cadmium on the other hand was elevated the most in S7 which is rice cereal. This finding corroborates with previous studies (Tchounwou et al., 2012, Parker et al., 2022 and Bair, 2022) which indicated that rice is a major source of cadmium.

In Figure 9, arsenic levels were generally low in all food types except in S7 which is rice cereal (0.1ug/g). This finding agrees with Parker et al., (2022) that found the highest levels of arsenic in rice cereal. Arsenic is naturally present at high levels in groundwater and is highly toxic. Since rice is naturally a semi-aquatic plant, this could explain the reason why it is the food product with the highest concentration of arsenic in this study.

Heavy metal contents of baby foods are of increasing and growing concern manly because of the need to continuously monitor the exposure of infants and toddlers to these elements from their diet. Contaminated food remains a major source of dietary exposure in babies and young children (Muntean et al., 2013). In this study, rice and sweet potato were the two main plant-based food products that had concentrations of evaluated metals exceeding the minimal risk levels in the different food types. These two food types have been shown to be basic staples in all food diets and care must be taken in how often children consume these plant-based products.

Arsenic and nickel also appear to be the two elements whose levels must be seriously monitored. This is because cereals and cereal based food products continue to be staples in infant and toddler diets. Despite the many studies that have raised concerns on the presence of arsenic in rice (Lai et al., 2015 and Hojsak et al., 2015,) rice continues to be used as a major staple in many baby foods such as teething and has been listed a major contributor of dietary arsenic exposure in children. Daily consumption of rice should be avoided to reduce the risk of children exposure to arsenic through this chain.

CHAPTER 5

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Heavy metal toxicity remains a major threat to human health due to the several health risks associated with its chronic accumulative nature. In this study, it is evident that plant-based products were the most contaminated of all the food types, having concentrations ranging from trace amount to higher levels. Although, most of the metals do not exceed the proposed MRL standard used in this study, it is important to note that heavy metals were still observed at detectable levels and some metals like arsenic and cadmium are potentially toxic even at extremely low concentrations of 0. 01 ug/g and 0.005 ug/g respectively. The toxicity of these heavy metals depend largely on the amount ingested and their bio-accumulation amidst other factors such as health status, age, diet and lifestyle, and for a vulnerable population like little children, extra caution has to be taken since there are still many unknown links between many health issues and behavioral disorders like autism and attention deficit/hyperactivity disorder (ADHD)

Since plant-based food products were observed to be the most contaminated in this study, this provides additional information that could guide baby food manufacturers so they could pay closer attention to where they source their raw materials from. Understanding this will help to strictly monitor food chain contaminations coming from anthropogenic sources and ensure the desired product quality.

The essential micro elements discussed in this literature such as chromium and nickel should be closely monitored since a lot still remains unknown about the link between their toxicity and disease disorder development. Also, nickel as a toxic metal should be regulated and monitored since studies have shown that although their levels are generally low in the environment, man-made activities involving the use and production of nickel industrially is gradually contributing to their increased levels in the environment. It is not safe to ignore setting a clear safe oral limit level for this metal, because some of the major concerns relating to the properties of toxic metals are their long persistence, stability in living systems and longer soil residence time. Ignoring the well of information we have about anthropogenic release of heavy metals could lead to incidences like the Itai-itai disease which was a chronic exposure.

Overall, anthropogenic sources of toxic metal release should be strictly monitored and modern agricultural products like sewage effluents reported as being used for irrigation purposes in farming should be highly discouraged. These are pathways to cutting off the soil to food crop chain of contamination which could lead to continuous challenges of toxic metal in food products, especially baby food.

The Closer to Zero Plan established by the FDA should implement setting clear maximum limits that are universal, updated in a timely manner and strictly encouraged to be followed by all baby food manufacturers, especially for nickel, cadmium, zinc, and lead. Leaving baby food manufacturers with the sole responsibility of defining their reference limits will always result in conflicting values that will keep allowing baby food heavy metal contaminations to pass globally. Monitoring food safety effectively remains a challenge for the developed and developing countries and is dependent on the combined efforts of the government, regulators such as the FDA, farmers, academia, food producers and suppliers. The success of this plan depends on studies such as this, providing enough scientific data that can help the FDA in fully implementing this plan.

APPENDIX A

TOTAL METAL CONTENT IN THE TEN FOOD SAMPLES ANALYZED IN TRIPLICATES EXCEPT FOR S1 AND S5 WHICH WERE ANALYZED IN DUPLICATES DUE TO A DIFFERENCE IN INGREDIENT FORMULATION

Lab Code	Sample Name	Al ug/g	Cr ug/g	Ni ug/g	Zn ug/g	As ug/g	Cd ug/g	Pb ug/g
S1-1	Parent's Choice Sweet Potato and Turkey Baby food puree, stage 3	0.2594	0.0539	0.3632	1.6266	0.0104	0.0027	0.0046
S1-2	Parent's Choice Sweet Potato and Turkey Baby food puree, stage 3	0.1745	0.0362	0.0772	1.6065	0.0112	0.0028	0.0036
S1-3	**Parent's Choice Sweet Potato and Cinnamon Vegetable puree, stage 2**	0.6300	0.6233	0.4339	1.2189	0.0086	0.0027	0.0047
S2-1	Beechnut pear, banana and raspberries fruities.	4.0334	0.2470	0.7467	1.3286	0.0114	0.0011	0.0012
S2-2	Beechnut pear, banana and raspberries fruities.	3.9530	0.0987	0.3304	1.3356	0.0115	0.0012	0.0012
S2-3	Beechnut pear, banana and raspberries fruities.	4.2820	0.0616	0.1655	1.3737	0.0131	0.0010	0.0017
S3-1	Earth's best organic sweet potato and apple baby food puree	2.1550	0.2304	0.1532	1.2316	0.0121	0.0031	0.0031
S3-2	Earth's best organic sweet potato and apple baby food puree	2.4831	0.3190	0.1462	1.4071	0.0143	0.0018	0.0035
S3-3	Earth's best organic sweet potato and apple baby food puree	2.8643	0.2511	0.1459	1.2724	0.0121	0.0018	0.0034
S4-1	Caprisun	0.3409	0.0159	0.0238	0.1293	0.0064	0.0001	0.0004
S4-2	Caprisun	0.3471	0.0128	0.0177	0.1516	0.0067	0.0001	0.0003
S4-3	Caprisun	0.2614	0.0092	0.0167	0.1351	0.0047	0.0001	0.0003
S5-1	Earth's best chicken casserole with vegetables and rice puree	0.7819	0.0442	0.0605	2.9416	0.0134	0.0038	0.0007
S5-2	**Earth's best BEEF Medley with vegetables puree**	1.3686	0.0974	0.0997	5.2681	0.0098	0.0085	0.0009
S5-3	Earth's best chicken casserole with vegetables and rice puree	0.8664	0.0513	0.0686	2.8576	0.0124	0.0047	0.0009
S6-1	Gerber's infant formula	0.3533	0.0312	0.0950	34.7324	0.0238	0.0002	0.0017
S6-2	Gerber's infant formula	0.3537	0.0310	0.0933	34.1536	0.0233	0.0001	0.0019
S6-3	Gerber's infant formula	0.3533	0.0335	0.0961	31.7728	0.0222	0.0001	0.0016
S7-1	Gerber's rice cereal	0.4583	0.0752	0.2466	69.9669	0.1059	0.0175	0.0015
S7-2	Gerber's rice cereal	0.4853	0.0600	0.2263	72.4947	0.0998	0.0172	0.0011
S7-3	Gerber's rice cereal	0.5324	0.0673	0.2310	66.1420	0.0989	0.0165	0.0013
S8-1	Similac advance infant formula	0.4548	0.0211	0.0589	31.9704	0.0120	0.0002	0.0007
S8-2	Similac advance infant formula	0.3944	0.0433	0.0612	29.5040	0.0117	0.0001	0.0007
S8-3	Similac advance infant formula	0.3991	0.0453	0.0884	29.2680	0.0117	0.0002	0.0007
S9-1	Great value syrup	0.1081	0.0136	0.0171	0.0845	0.0087	0.0001	0.0005
S9-2	Great value syrup	0.0855	0.0095	0.0145	0.0579	0.0087	0.0002	0.0003
S9-3	Great value syrup	0.0628	0.0080	0.0129	0.0465	0.0073	0.0002	0.0003
S10-1	Gerber's beef and gravy baby food	0.2444	0.0186	0.0192	22.1397	0.0080	0.0003	0.0004
S10-2	Gerber's beef and gravy baby food	0.2813	0.0177	0.0198	24.4565	0.0088	0.0007	0.0006
S10-3	Gerber's beef and gravy baby food	0.2878	0.0315	0.0209	24.4849	0.0106	0.0002	0.0005

APPENDIX B

MEAN VALUE CONCENTRATIONS OF THE HEAVY METAL CONTENTS OF THE 10 FOOD TYPES

Food type	**(Al) µg/g mean (SD)**	**(Cr) µg/g mean (SD)**	**(Ni) µg/g mean (SD)**	**(Zn) µg/g mean (SD)**	**(As) µg/g mean (SD)**	**(Cd) µg/g mean (SD)**	**(Pb) µg/g mean (SD)**
S1	0.217 (0.060)	0.045 (0.012)	0.220 (0.202)	1.617 (0.014)	0.011 (0.001)	0.003 (0.000)	0.004 (0.001)
S2	4.089 (0.172)	0.136 (0.098)	0.414 (0.300)	1.346 (0.024)	0.012 (0.001)	0.001 (0.000)	0.001 (0.000)
S3	2.501 (0.355)	0.267 (0.046)	0.148 (0.004)	1.304 (0.092)	0.013 (0.001)	0.002 (0.001)	0.003 (0.000)
S4	0.316 (0.048)	0.013 (0.003)	0.019 (0.004)	0.139 (0.012)	0.006 (0.001)	0.000 (0.000)	0.000 (0.000)
S5	0.824 (0.060)	0.048 (0.005)	0.065 (0.006)	2.90 (0.0059)	0.013 (0.001)	0.004 (0.001	0.001 (0.000)
S6	0.353 (0.000)	0.032 (0.001)	0.095 (0.001)	33.553 (1.5689)	0.023 (0.001)	0.000 (0.000)	0.002 (0.000)
S7	0.492019 (0.0375)	0.067 (0.008)	0.235 (0.011)	69.535 (3.198)	0.102 (0.004)	0.017 (0.001)	0.001 (0.000)
S8	0.416 (0.034)	0.037 (0.013)	0.069 (0.016)	30.247 (1.497)	0.012 (0.000)	0.000 (0.000)	0.000717 (3.457)
S9	0.085 (0.023)	0.010 (0.003)	0.015 (0.002)	0.063 (0.020)	0.008 (0.001)	0.000 (0.000)	0.000 (0.000)
S10	0.271 (0.023)	0.023 (0.008)	0.020 (0.001)	23.694 (1.346)	0.009 (0.001)	0.000 (0.000)	0.001 (0.000)

- SD refers to the standard deviation
- µg/g =mg/kg=ppm

APPENDIX C

AGENCY FOR TOXIC SUBSTANCES AND DISEASE REGISTRY WAS USED IN THIS STUDY

MINIMAL RISK LEVELS (MRLs)

April 2019

MRLs in draft Toxicological Profiles are provisional.

See bottom of table for more information.

https://wwwn.cdc.gov/TSP/MRLS/mrlslisting.aspx#

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