Fall 12-18-2015

Arsenic, Cadmium, Copper, and Zinc Levels in Crayfish from Southwest Louisiana and Atchafalaya Basin

E. Gerald Hebert
University of New Orleans, tboy11@cox.net

Arsenic, Cadmium, Copper, and Zinc Levels in Crayfish from Southwest Louisiana and the Atchafalaya Basin

A Thesis

Submitted to the Graduate Faculty of the
University of New Orleans
in partial fulfillment of the
requirements for the degree of

Master of Science
in
Earth and Environmental Science

by

E. Gerald Hebert

B.G.S., University of Louisiana at Lafayette, 2012

December, 2015

Acknowledgment

I would like to acknowledge my thesis advisors, Dr. Patricia Williams and Dr. Mostofa Sarwar. The leadership and interest shown by Dr. Williams and Dr. Sarwar were incredibly unique and advantageous in this endeavor. They are dedicated educators who embrace student success and the dynamics thereof. I would also like to acknowledge my thesis committee members: Dr. Tumulesh Solanky, Chair of Mathematics at the University of New Orleans, and Dr. Mark Zappi at University of Louisiana at Lafayette, Dean of Engineering. My heartfelt gratitude also goes to Mr. William Holmes, Director of Analysis and Process Chemistry who guided my research. I would also like to acknowledge my wife Martha and my son Gerald for their encouragement with this project.

E. Gerald Hebert

Table of Contents

(Table of Contents Cont.)

List of Figures

List of Tables

Hypothesis

Man-made ponds and the Atchafalaya Basin are crayfish habitats that may be influenced by heavy metal contamination from pesticides, insecticides, herbicides, fungicides, and fertilizers. Research suggests that certain products laden with arsenic and cadmium are carcinogens. The research herein is designed to identify, quantify, and compare heavy metal contamination of ingested arsenic, cadmium, copper, and zinc concentrations in crayfish tails and intestines consumed by humans in specific south Louisiana locales. The locales tested were chosen to reflect the heart of the crayfish industry, and had not previously been tested for heavy metal contamination.

Abstract

Heavy metal contamination in food is a worldwide concern. Man-made ponds are domestic sites in the production of Procambarus clarkii and Procambarus zonangulus, two edible species of crayfish. Ponds may be constructed in former sugar cane or rice fields. Crayfish farming is an ancillary seasonal business within the rice-growing season. The use of products to control insects, pests, and weeds in rice and sugar cane production, may cause an accumulation of heavy metals in the crayfish tail within pond structures. Arsenic, cadmium, copper, and zinc are heavy metals that are absorbed through the roots of and distributed through rice products. Metabolites associated with rice products are absorbed in the human body. Research suggests that metabolites associated with heavy metals cause disease in animals and humans.

Keywords: Methylation, Metabolites, Mean, Pesticides, Fertilizer

Chapter 1 – Introduction

Arsenic poisoning is the greatest single cause of ill health in the world today (WHO, 2012). The Environmental Protection Agency (EPA) states that arsenic is a naturally occurring element in soil and water and may be a source for absorption of arsenic by plants. There are no federal regulations limiting soil arsenic levels in the US. The EPA superfund risk model gives a value of 0.43 ppm total soil ingestion exposure of arsenic for a cancer risk of 1 in 10^6 (Duxbury and Zavala, 2012). Arsenic from hydrothermal veins and igneous rock bodies are associated with cobaltite, arsenopyrite, and arsenic products. Erosion from land use and smelting plants are sources of arsenic. Mineral processing and various domestic products such as paint and preservatives are also sources of contamination. Groundwater associated with geothermal activity is sometimes arsenic-rich (Rahman, et al., 2012).

The average level of arsenic is 5 ppm in U.S. soils, whereas in other countries it can vary from 10-20 ppm from agricultural use. Arsenic is the twentieth most common element in the earth's crust, entering the environment usually by volcanic or industrial activities. Arsenic is also present in 200 known mineral species. Mean air concentrations of arsenic are in the 0.02-4 ng/m^3 in remote areas and from 3-200 ng/m^3 in urban areas. Background or mean soil levels of arsenic can range from 1 – 5 mg/kg to a high of 40 mg/kg. Foods usually contain less than 0.25 mg/kg, depending on anthropogenic and pesticide usages in the tested soils (World Health Organization, 2001). Arsenic will adsorb to particulate matter and sediment, existing as arsenic As^{5+} and As^{3+} in two oxidation states. Arsenic sulfide (As_2S_3) exists in the oxidizing state and Arsine (A_SH_3) under reducing conditions. According to the Agency for Toxic Substances and Disease Registry (ATSDR), airborne arsenic particulate matter is oxidized by microbial action to nonvolatile species, and settles back to the ground (2006).

Toxicity occurs with a series of reactions that result in the reduction of inorganic Arsenate (As^5) to Arsenite (As^3), methylation to Monomethylarsonic acid (MMA^V), reduction to Monomethylarsonous acid (MMA^3), and methylation to Dimethylarsinic Acid (DMA^5). Arsenic is recognized as one of the most global toxicants, causing acute and chronic health effects inclusive of cancer. Arsenic is an analog of phosphates and can replace phosphates in many critical cellular functions, producing cellular toxicity or mutagenicity. Bio-accumulation is inevitable in humans and animals. (ATSDR, 2007)

Arsenic undergoes reactions in the environment including oxidation-reactions, precipitation, bio-transformation and ligand exchanges. Conditions are influenced by Eh (the oxidation-reduction potential), pH, metal sulfide, sulfide ion concentrations, iron concentrations, temperature, salinity, and distribution and composition of the biota (ATSDR, 2007).

Mining or manufacturing areas have higher arsenic concentrations. The standard level of arsenic in drinking water adopted by the EPA on January 23, 2006 is 10 µg/L, replacing the previous level of 50 µg/L. Arsenic occurs naturally in coal and oil, thus arsenic is released into the atmosphere as a result of coal and oil-fired power plants. Cotton ginning is a source of arsenic emissions released to the atmosphere, when cottonseeds are removed from raw cotton. U.S. emissions to the atmosphere were estimated to be 3,300 metric tons per year between 1979 and 1986 (ATSDR, 2007). Industrial regulations of coal burning plants have reduced arsenic emissions since the 1980s. At one time, copper smelting and coal combustion accounted for 65% of anthropogenic emissions, approximated to be about 12,000-25,000 metric tons a year with natural sources depositing about 11,000 – 23,500 tons per year (ATSDR, 2006).

Processing plants and factories create abundant commercial run offs, and arsenic exposure in rivers, lakes, streams, and ponds adversely affecting ground water. Pesticides and

fertilizers also release arsenic to the soil. Roxarsone, a drug added to poultry feed to improve production, also contains organic arsenic (ATSDR, 2006).

The iron content of the soil affects arsenic absorption. Arsenic absorbed by iron and manganese is released and reduced especially during flooding conditions. Abundant nutrients available for microbial action may result in dissolution. Soil drying after flooding increases arsenic action through mineralogy or iron absorption (ATSDR, 2007).

The oxidation states of arsenic exist as As^{5+}, As^{3+}, arsenic (As^{o}), and A_SH_3. Generally, the inorganic species is more toxic than the organic, depending on concentration and speciation. As^{3+} is more toxic than As^{5+}, whereas dimethylarsinous acid (DMA^{III}) and monomethylarsonous acid (MMA^{III}) are more toxic as metabolites than the parent compounds of As^{3+}. The best metabolite concentration indicator is an arsenic urine sample test. Monomethylarsonic acid (MMA (V)) and dimethylarsinic acid (DMA(V)) -- both indicators of recent exposures -- are often misidentified. Recent studies revealed monomethylmonothioarsonic acid ($MMMTA^{V}$) and dimethylmonothioarsinic acid ($DMMTA^{V}$) in red blood cells and possibly in other cells, pose 10-fold less toxicity than MMA^{III} and DMA^{III}. These metabolites are associated with sulfur-containing methylated inorganic exposures. When testing urine samples for arsenic exposure, they are often misidentified as MMA^{III} and DMA^{III} in exposed individuals, sometimes confounding results, although considered deleterious to human health as carcinogens and cancer causing substances (Van de Wilde et al., 2010).

Inorganic arsenic is most abundant in several countries; Argentina, Chile, India (West Bengal), Mexico, and the United States of America as well as Bangladesh, with half of the total population at risk with the extremely high arsenic contamination in the drinking water supply in

tube wells. One estimate from these locales showed 9100 deaths and 125,000 disability-adjusted life years due to arsenic exposure (Duxbury & Zavala, 2012).

Smoking cigarettes introduces pesticide-laden tobacco into the human body. Lung cancer is directly associated with cigarette smoking. Arsenic is methylated and eliminated from the body by the urinary process. The methylation process is inhibited by lowering arsenic elimination from the human body. Smoking acts synergistically with arsenic exposure causing DNA damage in the lungs. Smokers have a higher risk of lung, bladder, and skin lesions associated with cancer as well as heart disease (Lazarevic et al., 2012).

The World Health Organization's arsenic guidelines established the tolerable intake level of arsenic in 2010. The scientific evidence by the Joint Food and Agriculture Organization of the United Nations (FAO) and the WHO also determined the lower limit benchmark dose of arsenic from epidemiological data to be 3.0 µg/kg body weight per day and 2-7 µg/kg body weight per day, based on the range of estimated total dietary exposure. Ingesting levels below the lower benchmark reduces the incidence of lung cancer below the minimum detectable limit (BMDL 0.5). The previous provisional tolerable weekly intake (PTWI) of 15 µg/kg body weight BMDL 0.5 (equivalent to 2.1 µg/kg body weight per day) for inorganic arsenic is no longer appropriate, and the benchmark was withdrawn. The WHO tolerable limits of drinking water and arsenic levels are acceptable; no new tolerable guidelines have been established. Ten µg/L or below is the acceptable WHO guideline for arsenic in drinking water (Duxbury and Zavala, 2012).

Chapter 1.1 – Procambarus clarkii and Procambarus zonangulus

This study tested accumulation of arsenic in Procambarus clarkii (Red Swamp crayfish) and Procambarus zonangulus (White River crayfish) in crayfish ponds converted from rice (Oryza sativa) and sugarcane fields in central and southwest Louisiana, and in the Atchafalaya Basin (Anderson et al., 1997).

Crayfish farming incorporates the use of fertilizers, pesticides, herbicides, and fungicides creating a possible accumulative effect of arsenic in the particular pond from which the sample of crayfish species were procured. Arsenic is taken up in the rice shoots grown in the particular pond for food. Afterwards, research suggests the crayfish ingest the rice containing arsenic as food, and are then consumed by humans (Avery and Lorio, 1999).

Arsenic is methylated and bio-transformed in marine and fresh waters, creating toxic metabolites. Research suggests that some forms of arsenic metabolites like arsenobetaine (AsB) are innocuous, due to a covalent bond that prevents toxic metabolite formation. It is a form of organic arsenic found in seafood that is excreted in the urine (Devesa et al., 2005). However, there are reports that show at very high concentrations some arsenobetaine will be metabolized in humans (Choi, 2010).

Farmed crayfish in converted ponds may be susceptible to inorganic arsenic contamination and accumulation from fertilizers, insecticides, pesticides, herbicides, and fungicides. Previous contamination from sugarcane, corn, or rice field crops over the years may have an impact on arsenic contamination and accumulation in the ponds. Humans consume crayfish; thus, arsenic will bio-accumulate. Some arsenic metabolites are known cancer-causing substances. The research design attempts to quantify arsenic content in pond-raised crayfish, as

compared to wild-grown crayfish, possibly determining a higher Minimum Risk Level (MRL) contamination in humans (Avery and Lorio, 1999).

Chapter 1.2 – History of Growing Crayfish

A commercial source of seafood in Louisiana, crayfish date back to the 1800s to the old French market of New Orleans. In 1940 a commercial fishery for wild crayfish harvested in the Atchafalaya swamp, and to this day provides a large catch each year. The 1950s showed an experimental industry with new pond acreage continually increasing with time. Crayfish ponds vary widely in shape and size. Ponds are normally 10-20 acres, with larger producers managing up to100 acres. Louisiana is the largest producer of crayfish in the US. Crayfish are not formula-fed, but instead are allowed to ingest rice, sorghum-sun grass, or other natural vegetation. Farmers rely on un-harvested crayfish for the next year's brood stock (Avery & Lorio, 1999).

Varying production yields affect profits from year to year. Consultation from local higher education entities, such as LSU Co-Operative Extension aids in decision-making relative to crayfish farming and its success (Avery and Lorio, 1999).

Thirty-two species of crayfish have been identified in Louisiana. The two most important economic species are the Red Swamp and White River crayfish. Both can be grown in the same pond, although the Red swamp crayfish makes up approximately 80% of the annual catch. Adult Red Swamp crayfish are distinguished from the White River species. The Red Swamp crayfish has two halves of the carapace meeting to form a thin line. White River crayfish exhibit a separation between the two halves of the carapace and lighter colored walking legs. Red swamp crayfish have a vein line on the underside of the tail. The White River crayfish does not have a vein line (Avery and Lorio, 1999).

After mature crayfish mate, the female burrows deep into the levee from 4-6 inches above the water level. After an ovarian development period of 2-5 months, anywhere from 100 to 700 eggs are deposited and attached to swimmerets on the underside of their bellies. Eggs

usually hatch in 2 – 3 weeks under ideal conditions, but may take 3 – 4 months in winter. Red Swamp crayfish reproduce up to twice as often as White River crayfish (Avery and Lorio, 1999).

Crayfish production peaks in the spring weather during April through June. Crayfish burrow deep into the ground during dry months seeking moisture, and returning to the surface primarily during the fall, when the rainy season begins. Crayfish may be found 6-8 feet deep below the earth's surface in some cases. Wetlands and surface locations fill up with water during the rainy season beginning in autumn, and the crayfish return to the surface, depositing their eggs and offspring in open water, setting the stage for the new season's harvest. Availability of crayfish depends on weather and rain, creating a new cycle of production (Avery and Lorio, 1999).

The Atchafalaya Basin, a long time haven, provides the natural habitat for wild crayfish production. A short season prevails in the wild every year, although a catch is eminent under normal weather conditions. The Atchafalaya Basin is the cradle of the crayfish industry, dating as far back as early Native Americans, who introduced the European settlers to the crayfish, a delicacy as well as a food source for survival (Avery and Lorio, 1999).

In the late 1960s, LSU and A & M College started assisting aquaculture with crayfish farming, and still provide the foundation and structure of crayfish farming today. Some farmers rotate the rice and crayfish crops from year to year, although most farmers leave the rice in the fields as leftover for the crayfish. The full term of crayfish from hatching to consumption is 90 days (Avery and Lorio, 1999).

Crayfish, like crabs and lobsters, occasionally lose their hard shell, and can double in size every time they molt. October flooding summons female crayfish to resurface and to deposit their eggs. Early spring is the greatest period of consumption (Avery and Lorio, 1999).

Barometric pressure activates the production of crayfish during rainy periods. The pond crayfish season starts in November in a warm year, and as late as February in a cold year, lasting as late as July (Avery and Lorio, 1999).

Studies associated with heavy metals performed in the past reveal information relative to arsenic contamination in areas of natural habitats of crayfish. Abdominal muscles and hepatopancreatic tissue were analyzed separately for lead, mercury, and cadmium, whereas arsenic was discovered in defoliating agents from the use of arsenicals (now banned) in pesticides. Tests showed as much as 380 mg/kg of arsenic due to contamination by metal processing in industry, and 625 mg/kg of arsenic from arsenical pesticides (Finerty et al., 1990).

Accumulation of arsenic occurs in the gills, exoskeleton, hepatopancreatic viscera, and abdominal muscles. Concentrations of heavy metals in crayfish organs were highest in the gills and hepatopancreas, which serve as biomarkers of pollution (Anderson et al., 1997; Tunca et al., 2013). Note that in a particular analysis of male and female crayfish, the female showed less bioaccumulation of nickel and arsenic in gill tissues, as compared to males in the same species. The gills and the exoskeleton had the highest concentration of heavy metals (Tunca et al., 2013).

Chapter 1.3 – Sugar Cane

Louisiana produces 42% of the sugar cane produced in the US. In the 1720s, the French brought sugarcane plants to Louisiana. After initial failed attempts to produce sugar, commercial scale production of sugar cane began in 1796 with 100,000 pounds. In 1891, Louisiana production was 550 million pounds of sugar. (Gianessi, 2009)Approximately 455,000 acres yields about 21 billion pounds of sugar valued at $260 million dollars per year. Louisiana sugar mills produce 2.8 billion lbs of raw sugar annually (Gianessi, 2009).

The sugarcane borer is the most damaging insect to the sugarcane industry. Only 10% of the pests survive the winter, hibernating in scraps of cane, grass, cane stubble, and planted cane. Natural mortality factors controlling the borer population are cold weather and rain. The larvae eggs hatch in spring and immediately begin foraging for survival. A 1925 severe infestation of the borers destroyed one-third of the total crop in the state. Parasites were introduced to control the borer population as done in the Caribbean Islands. Most of the parasites died off, and only one species was left to control about 4% of the borer population from year to year (Gianessi, 2009).

Arsenals were first used in the control of borers, but were not effective. The larvae would push the arsenals to the side before feeding, and less than 20% were destroyed. Research created genetic changes on the leaf-sheath as well as the cane stalk that prevented larval movement, and hardened of the internodes, reducing penetration of the canestalk and feeding of the borer. The inorganic compound cryolite, a naturally occurring mineral, was introduced into the cane fields in the early 1920s for borer control. Fire ants were also introduced as a predator to the borer in sugarcane fields, but the fire ant populations later had to be controlled using heptachlor. Heptachlor was replaced several times, first with endrin, an organochlorine, then with azinphos-

methyl, an organophosphate in 1964 (Gianessi, 2009). Botanical compounds of Ryania, a mixture of alkaloids made from a plant root, were replaced with synthetic organic chemicals, improving the sugarcane borer control program in Louisiana. Applications were reduced from 12 to 2 due with the introduction of tebufenozide, a much more effective insecticide (Gianessi, 2009).

Chapter 1.4 – Background Research

The research herein on tails and intestines is being compared to previous research on arsenic associated with crayfish from petroleum-based effluents deposited in a Louisiana waterway by a manufacturing plant. Arsenic, lead, chromium, and copper were found, although arsenic and cadmium were lower in content. The hepatopancreas and gills showed significant accumulation whereas copper was identified but did not show any significant change during the research study. Abdominal muscles showed little accumulation of arsenic, due to a lower percentage in the sediment tested as a primary factor in the wetland research. This research paralleled sediment concentrations according to test results in the wetlands study with the pollution of hydrocarbons containing heavy metal concentrations (Anderson et al., 1997).

Heavy metalloid research was conducted on the exoskeleton, gills, hepatopancreas, and abdominal muscle tissues of the Astacus leptodacylus specimen at a wetland site in Turkey. Eleven metals were tested including aluminum, chromium, manganese, cobalt, nickel, copper, molybdenum, silver, cadmium, mercury, lead, and arsenic. Correlation trends were established and evaluated by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). Elements were evaluated in tissue samples of both heavy metal and metalloid concentrations (Tunca et al., 2013).

An assessment of the human health risk associated with consuming the herbicide, 2,4-dichlorophenoxyacetic acid and monosodium methanearsonate was performed. The exposed crayfish showed an exposure dose at the high end of consumption that was approximately twice the reference dose for arsenic. The cancer risk averaged approximately 7 extra tumors in a population of 10,000. Six extra tumors in a population of 10,000 resulted from a lifetime

consumption of crayfish, exposed to the herbicide mixture with or without surfactant. (Green and Abdelghani, 2004)

Chapter 2 – Arsenic

The EPA states that arsenic is a naturally-occurring element in soil and water. The EPA also assumes there is no safe level of exposure to inorganic arsenic. Rice seems to be the biggest provider of arsenic in the organic form. Limiting intake of rice products is a good defense against arsenic. Testing showed that total inorganic arsenic levels were always higher for brown rice than white rice (Snyder & Slaton, 2001). Research suggests that cancer is prevalent in south Louisiana, and suggests a correlation between cancer rates and the consumption of cereal and rice products (66% through consumption of plant sources, and 33% from animal sources) (Luckett et al., 2012).

Sixty-five rice products have been analyzed and ranked categorically as 100 substances that are Group 1 carcinogens. Arsenic is known to cause bladder, kidney, and prostate cancer in humans. The US is the leads agriculture and industry with 1.6 million tons of arsenic used since 1901. Lead arsenate insecticides caused bioaccumulation in soil and water, although banned in 1980. Fertilizers made from poultry waste may contaminate crops with inorganic arsenic. (Bellows, 2005)

Research suggests deleterious effects from arsenic exposure established by The Food and Drug Administration (FDA) with a limit of .01 mg/L, in the US as the acceptable limit in drinking water. Disease and effects are more profound in areas of unregulated drinking water supplies. Use in paints and wood preservatives is common in home construction and industry, creating a potential dust particle inhalation factor from degradation of wood products during construction, remodeling, and demolition. Arsenic ingestion from water supplies, inhalation of dust particles, and food supply contamination are the primary means of disease contraction in humans and animals. Education and mechanical remediation can lesson potential contamination.

In-home water filtering systems can remove the toxic element from drinking water. Proper handling of food supplies can reduce the intake of arsenic as well. Problems with arsenic are becoming less common worldwide because of improved precautions (Consumer Reports, 2012).

Arsenic was used as a chemotherapeutic in the treatment of infectious diseases such as syphilis and typanosomiasis. The therapeutic attributes are far outweighed by its toxic effects with higher doses and accumulation due to food and water intake (Kaur et al., 2011; Lundh et al., 2010).

Aquatic environments are the recipients of household and industrial discharges with arsenic, as well as spraying pesticides on gardens and farmlands. Aquatic pollution adds to the concentration and bioaccumulation of plant absorption as well as accumulation of the metalloid in fish in polluted waters, which are both eaten by humans. Disposal strategies and discharges are sometimes deleterious to the environment, increasing the arsenic levels in ground water supplies. Birds that eat fish with high concentrations of arsenic will die from the fish decomposition containing high concentrations of arsenic in their bodies (Kaur et al., 2011; ATSDR, 2007).

Arsenic is a known neurotoxin with natural body mechanisms aiding in the excretion and extraction of the substance. Urinal, stool, and breath extraction methods aid in the discharge of arsenic. Excessive intake lodges in tissues, inhibiting cellular activities. Not only interfering with homeostasis, arsenic intensifies the onset of lung, liver, and lymphatic cancers in the human body. Higher concentrations cause infertility and miscarriages in women, infection resistance, and brain damage in both men and women. The National Toxicology Program considers arsenic DNA damage a human carcinogen. The allowable MRL associated with arsenic is 10-50 ppb as

stated by the EPA. The MRL for oral acute ingestion is .005 mg/kg/day and .0003 mg/kg/day for chronic ingestion (Kaur et al., 2011; Lundh et al., 2010).

Transplacental arsenic exposure caused an increase in estrogen receptor-a (ER-a) transcript and protein levels in the lungs of females. Arsenic exposure also caused an insulin growth factor (IGF), an Estrogen Receptor (ER) catalytic response, and pulmonary oncogenic process activation in the fetal lung. The data set and research shows evidence of arsenic-induced aberrant ER signaling in early life stages and genetic programming, causing tumors in the lung later in adulthood (Shen et al., 2007).

DMA^V, a metabolite of ingested inorganic arsenic is associated with urinary bladder cancer in rats and a multi-tumor promoter in mice and rats. Research shows that lung adenoma-carcinomas increased from 0 % to 20% in C3H mice with gestational inorganic arsenic inducing pulmonary carcinogenesis promoted by phorbol esters (croton oil) in male and female offspring. It acts synergistically as a tumor promoter. Succinct evidence of trans-placental factors indicative of arsenic crossing the rodent and human placenta, acting on fetal development, shows the lung as a target of arsenic carcinogenesis (Shen et al., 2007).

Many parts of the world less regulated by government agencies, such as the FDA in the US, must address contamination levels as causations of bladder, skin, and lung perturbations. Research suggests that individuals subjected to levels of 300 ppb have a 1 in 300 chance of a cancer diagnosis. Amazingly, one can ingest that much organic arsenic in a half-cup of rice or a glass of fruit juice, much higher than the MRLs as compared to a glass of pure drinking water (Lundh et al., 2010). According to some research mediums, the food industry denies the presence of organic arsenic forms found in rice or fruit juices (Lundh et al., 2010).

Arsenic-based pesticides are often used in the rice growing process. The bioaccumulation of the generated rice product is arsenic laden with levels that abnormally affect homeostasis, possibly creating carcinogenic effects. The FDA is presently testing arsenic levels with statistical and concise analysis. The demand is a pronounced statistical approach to determine arsenic levels in consumable rice products and minimizing risks associated with intake. Acceptable naturally occurring arsenic levels or the Maximum Contaminant Level (MCL) is 0.01 mg/L. This level is considered safe for drinking water for long-term consumption, which is the actual Department of Health guideline for private water wells. Risk assessments monitor both organic and inorganic forms of arsenic testing mediums that do not usually differentiate between the two in reporting a concentration level, although inorganic arsenic is considered more deleterious to human health. A major source of ingested arsenic is a fish diet, providing a larger intake of the organic source or arsenobetaine. Some research suggests that arsenobetaine is much less dangerous as a cancer causing substance (Cubadda et al., 2012).

Higher exposures to inorganic arsenic in individuals living in high-risk areas are of concern. Instead of inorganic arsenic testing, metabolite analysis in urine is more reliable for determining exposure using speciation analysis. Metabolites are a better indicator of food and water arsenic contamination in people chronically exposed to the heavy metal. Dimethylarsinic acid (DMA) and monomethylarsonic acid (MMA) are ingested as arsenic and discharged as metabolites. Methylation capacity is measured by MMA and DMA proportions unless food rich in DMA or those that contain arsenic compounds are metabolized to DMA that would confound the results (Cubadda et al., 2012).

Inorganic arsenic is known as a human carcinogen and can produce cardiovascular disease as well as diabetes. Fetal and infant development is also impaired depending on

individual variations relative to arsenic metabolism. Cereal and grain foods for dietary intake may also be sources of arsenic, when not subjected to aquatic exposure. Rice and algae foods are known to have a higher content of arsenic well above the normal daily intake level (Roman et al., 2010).

As stated previously, intake of inorganic arsenic is most important with urinary arsenic testing and observance. Results are less substantial unless DMA and MMA are the realized measures associated with physiological arsenic discovery (Cubadda et al., 2012).

Chapter 2.1 – Arsenic Contamination in Other Countries

Arsenic contamination exceeding threshold limits can inhibit and harm homeostasis. Cardiologic studies or non-cancer effects show that high levels of arsenic over the allowable level of 50 µg/L in drinking water may also cause cancer (Kaur et al., 2011).

Bangladesh, Chile, and the West Bengal region of India have a pervasive problem with organic earthen arsenic being released into drinking water supplies, causing bioaccumulation and its resultant effects in pregnant mothers. Incidences of chronic cough, bronchitis, shortness of breath, and obstructive or restrictive lung disease are clinical conditions produced by arsenic, both carcinogenic and non-carcinogenic. Rat embryos exposed to arsenic up to day 18 of gestation with 500 ppm altered gene expression in 59 genes and 34 proteins, and altered the B-catinin and c-myc pathways (regulators of lung development that play a role in lung cancer and chronic lung diseases) (Petrick et al., 2009).

Machinery mechanisms are the answer in Bangladesh, as new technology is developed that will facilitate removal of the element. In just one district, 575 arsenic plants are having this new technology installed. Innovations include filtering systems capable of handling high water volumes. The plants are designed to remove arsenic above the allowable level (above a 3 µg/L) in the water supply ingested by the immediate population. Water testing reveals the arsenic levels after plant diffusion and removal of the heavy metal. Investigations show skin lesions in approximately 1200 patients with ongoing research, a decrease in the number of affected individuals (Chakraborti, 2001).

The major source of arsenic contamination in Chile's rivers is industrial pollution, dating back as far as 1971. Heart issues obtained for speciation analysis from arteries and veins with

coronary heart disease help researchers to understand long-term effects from arsenic exposure on vascular and cardio-vascular diseases (Roman et al., 2010; Shen et al., 2007).

Lung disease resulting from non-malignant adenomas in utero provides evidence of arsenic-laden drinking water in many demographics, and is signaled by lowered respiratory function, including shortness of breath. Lung cancer seems to be the most common cause of death; adenomas may become cancerous later in life. Prolific levels upwards of over 10 ppb and above are causations to the deleterious effects to humans and animals. Crepitations, chronic bronchitis, and bronchiectasis have been noted as well (Dauphine, et al., 2011).

Early life exposure from drinking water in Chile caused 11.5% lower forced expiratory volume in 1s (FEV1) ($P = 0.04$), 12.2% lower forced vital capacity (FVC) ($P = 0.04$), and increases in breathlessness (prevalence odds ratio [POR] = 5.94%; confidence interval [CI] 1:36 – 26.0). Research suggests that irreversible respiratory exposures to arsenic mimics smoking through adulthood, a known pulmonary complication. Northern Chile was laden with high arsenic levels, as much as 870 µg/L, until a mechanical arsenic removal plant was installed. Levels are being maintained at less than 10 µg/L (Dauphine et al., 2011). Chilean research suggests that pulmonary testing barometers of FVC and FEV with the POR above 1.0 are indicators of breathlessness, which identifies early life exposure to arsenic. The lung has a propensity to accumulate arsenic, but not as much as the liver and kidneys, which are major excretory organs (Dauphine et al., 2011).

Another disruption was estrogen signaling, evident in transplacental arsenic, inducing hepatic carcinogenesis. In a particular study, arsenic was noticed only in female mice as a trans-placental lung carcinogen. Human children exposed to arsenic in utero experienced increased lung cancer incidence as well as chronic disease later in life. Studies associated with developing

fetuses, cohort to maternal exposure to arsenic during day 8 to 18 of gestation led to multiple incidences of tumor formations in the lungs (Petrick et al., 2009).

Chapter 2.2 – Arsenic Methylation

White and red blood cells – aided by glutathione -- reduce As^{5+} to As^{3+} after being taken up in the blood stream. A portion of As^{3+} is methylated in the liver by enzymatic Methyl group transfer s-adenosyl methionine (SAM) to monomethylarsonic acid (MMA(V)) and dimethyl arsenate (DMA(V)). These metabolites are excreted readily; methylation is an important form of arsenic detoxification (ATSDR, 2009). Other research suggests that methylation is toxification rather than detoxification of inorganic arsenic. Trivalent methylated arsenic metabolites of MMA(III) and DMA(III) are created capable of interacting with cellular targets such as proteins and DNA (ATSDR, 2009).

Research shows that methylation decreases with higher concentrations of arsenic. Methylated arsenic species in urine is similar between siblings and parents, suggesting that arsenic methylation can be genetically linked. Increased retention of arsenic in soft tissue is amplified after methylation exceeds maximum capacity in the liver (ATSDR, 2009).

The percentages of excreted arsenic in urine are 50% dimethylated, whereas 25% is monomethylated, and the remaining is inorganic arsenic with some possible variations. In cases of higher urinary arsenic concentration, the methylated form is increased. In lower urinary totals, the predominant form is inorganic arsenic (ATSDR, 2009).

Chapter 2.3 – Arsenic Affects DNA and Genes

Postnatal arsenic exposures surpassing threshold accumulation levels, may act on DNA and can cause cardiac abnormalities, arteriosclerosis, and chronic pulmonary disease. Other genes of interest are extracellular matrix genes, cell motility genes, and those that regulate fetal growth. Aberrant airway remodeling is noted in many diseases including emphysema, asthma, idiopathic pulmonary fibrosis, tuberculosis, and bronchiectasis. Structural tissue changes from injury with deregulated repair, alters extra cellular matrix, and creates deposition in the airway wall (remnants of chronic inflammation) (Petrick, et al., 2009).

Arsenic exposure can lead to alterations in gene expressions by methylation which is long-term, and heritable in offspring from arsenic exposure. Due to methylation and bioaccumulation, embryonic development is altered, causing disease. Hyper-methylation of arsenic causes teratogenic factors, and gene expression alterations. Analysis shows alterations of MSH6, a protein recognized with DNA mismatches and mismatch repairs (Salnikow & Zhitkovich, 2008). The protein increases in arsenic-exposed animals, an indication of increases in repair. DNA repair proteins are also associated with apoptosis caused by arsenic exposures. Arsenic was also identified as a developmental toxicant in the rat lung because it can reduce body weight, fetal weight, and lung to body ratios as well as altering differentiation markers, causing changes in fetal growth and development. The result may be underdeveloped fetal lungs (Petrick et al., 2009).

Research suggests DMA(V), a metabolite of inorganic arsenic and a multi-tumor promoter, is retained longer in the lungs than in other tissues. Early transplacental exposures in both umbilical and maternal blood are similar, cause harmful effects after birth. A recent study in

Argentina showed less metabolized DMA in children than adults. This particular finding also showed higher death rates from lung cancer in early life (Dauphine et al., 2011).

The translocation of broken strands of DNA is linked to cancer and tumor proliferation. Apoptosis (programmed cell death) prevents tumor initiation and development. Apoptosis is the repair process to inhibited DNA cell structure. An example of DNA mix-ups are indicative of cancer and the translocation of broken strands of DNA in pairing of partners from the wrong chromosomes after breakage. When a heavy metal hits a DNA chromosome strand it splits, drifts apart, and searches for the missing partner. Sometimes the missing partner pairs to another unmatched chromosome strand becoming the precursor to mutations and cancer. Apoptosis or the programmed cell death process that prevents tumor initiation and development is the repair process to inhibited DNA cell structures, caused by Arsenic. Arsenic intensifies the onset of lung, liver, and lymphatic cancers in the human body. Higher concentrations cause infertility and miscarriages in women, infection resistance, and brain damage in both men and women. Arsenic exposure also caused an IGF, an ER catalytic response, and pulmonary oncogenic process activation in the fetal lung. (Shen, et al., 2007)

Chapter 2.4 – Research Mediums

Testing of chick embryos injected with sodium arsenate showed congenital malformations in day 8 to 9 of fetal gestation. A hamster injected with Sodium arsenate on day 8 of gestation in the implanted embryo undergoes axial orientation, rapid progression of developmental stages, somite differentiation, neural tube closure, visceral arch formation, limb bud formation, and embryonic heartbeat. Toxic levels in this particular study revealed that 30 mg/kg of arsenic would kill embryos in utero, with teratogenic effects occurring at 10 mg/kg. Urogenital anomalies were noticed with sodium arsenate levels in female and male subjects. Malformations were indicated in the uterus, ovaries, and testes, with unilateral and bilateral renal agenesis (Ferm, 1977). During human pregnancy, arsenic trioxide (As_2O_3) manifests itself to higher levels in the brain, kidneys, and liver crossing the placenta by day 8 of fetal gestation (Ferm, 1977; Miller et al., 2002).

Studies initiated with mice and tetradecanoylphorbol-13 acetate (TPA) as a cohort carcinogen causes hepatic carcinoma, as well as adrenal tumors. Female offspring showed an increase in epithelial ovarian tumors from arsenic exposure in lieu of TPA as well as hyperplasia of the uterus and oviduct. Gestational development is highly sensitive to carcinogenesis from arsenic exposure. Drinking water is a common cause of contamination, resulting in threshold limits of arsenic in humans. Mice born to maternally exposed females may show cancer effects after birth, well into adulthood. Evidence of 10-day transplacental arsenic exposure produced cancer effects in children (Waalkes et al., 2003).

DMA(V) is discharged through the bladder, thus cancerous bladder tumors are prevalent in mice with accumulation from chronically ingested arsenic-laden water. The most profound discovery to cancer was a cohort to DMA^V and prenatal arsenic, followed by DMA^V through

adult life. The incidence rate of cancer was relatively low (17%), because spontaneous renal tumors are rare in mice. Research also suggests that DMA(V), passing through the kidney as a biomethylation metabolite, promotes tumors in rodents as well as in human fetal development (Dauphine et al., 2011). Research suggests that maternal urine contains more MMA and As than DMA(V), suggesting that methylated species have shorter half-lives of elimination than inorganic forms (Hall et al., 2007).

Measures of blood arsenic metabolites, urinary arsenic metabolites, plasma folate, B-12 vitamins and homocysteine levels due to water concentrations across a wide spread demographics can affect arsenic concentrations. A particular study showed a strong correlation of 0.1-661.0 µg/L between maternal blood and cord total blood arsenic ($r = 0.84$; $p < 0{,}0001$). Transplacental exposure reveals a correlation between levels of arsenic exposure and lower IQs in children. Poor visual motor functioning was found in two groups of 6 and 10-year-old children, although no distinction was made between prenatal and postnatal exposure in those tested (Hall et al., 2007).

Folic acid enhances the methylation process. Prenatal folic acid supplements are necessary in areas of higher arsenic concentrations in drinking water, aiding in arsenic methylation. Folate and homocystines are especially necessary to arsenic methylation in fetal development. Folate deficiency may cause spontaneous abortions, placenta abruption, preeclampsia, and increased tissue retention in fetal gestation. If folate supplements are ingested and maintained throughout pregnancy, postnatal concentrations in newborns are much higher than the maternal pre-birth concentrations in mothers (Hall et al., 2007).

Since the liver is a site for DMA(V) biomethylation of inorganic arsenic, prenatal exposure, and fetal gestation could possibly prime hepatic events to carcinogenesis, through

inorganic arsenic biomethylation, thus promoting a product by hepatic metabolism derived from gestational development. Fetal arsenic-induced liver cancer was greatly magnified by DMA^V exposure into human adulthood and caused renal cancer in mice (Tokar et al., 2012).

Arsenic destroys human cells and results in apoptosis, indicative of threshold limits. As_2O_3 inhibits tumor causing apoptosis and cell death, for example, in breast cancer. Mechanistic studies of multiple cellular and molecular indices such as MTT assay, apoptosis ELISA assay, gene transfection, and invasion assays find reductions in cellular viability in arsenic oxide-treated cells in a dose-dependent cell, inherent in apoptosis in Notch 1 pathways. The Notch 1 pathways signal ligand-receptors that play a role in cell proliferation and apoptotic cell death, differentiation, invasion, angiogenesis, tumor metastasis, and cancer stem cell renewal in human breast cancer. Specific ligand and receptor genes have been identified in the actuality of Notch signaling pathways. Research suggests that Notch target genes and Notch signaling pathways are commonly associated with breast cancer. Notch 1,2,3, and 4 receptors as well as 5 ligands have been identified and associated with breast cancer. Another research medium has determined that high expression of Notch receptors and ligand observation is a weak indicator of breast cancer (Roman et al., 2010).

Arsenic becomes the therapeutic factor in arresting apoptosis with As_2O_3, a reagent used in the treatment of acute promyelocytic leukemia and other human cancers including breast cancer. Molecular mechanisms yielding success of As_2O_3 treatment is somewhat elusive, but is worthy of further research (Kile et al., 2012).

The problem becomes ubiquitous in nature due to inorganic arsenic exposure associated with cancer risks of neurological, craniological, respiratory, hepatic, and hematological diseases.

Inorganic arsenic is classified as a human carcinogen, especially in tests of fetal tissue. Research potentially links in-utero exposures to chronic diseases in adulthood (Kile et al., 2012).

Chapter 2.5 – Arsenic, Fetal and Embryonic Development

Blood concentrations of arsenic are too low and transient; some research suggests that blood concentration is not a biomarker for chronic arsenic poisoning. Total arsenic amount in urine has been in use as an indicator of recent arsenic exposure, because the kidney is the main route of excretion of many arsenic species (Mazumder, 2000).

DMA(V) is carcinogenic in mice and rats. Transplacental arsenic exposure produces internal tumors in mice offspring into adulthood. Research shows that the occurrence of lung adenoma-carcinomas increased from 0 % to 20% in C3H mice with gestational inorganic arsenic exposure, inducing pulmonary carcinogenesis promoted by phorbol esters (croton oil) in male and female offspring. Evidence of transplacental factors indicative of arsenic crossing the rodent and human placenta, acts on fetal development showing the lung as a target of arsenic carcinogenesis (Shen et al., 2007).

Incidences of chronic cough, bronchitis, shortness of breath, and obstructive or restrictive lung disease are lung perturbations targeted by arsenic, both carcinogenic and non-carcinogenic. Estrogen signaling, evident in the trans-placental arsenic, may induce hepatic carcinogenesis. In this particular study, arsenic was noticed only in female mice as a trans-placental lung carcinogen. Children exposed to arsenic in utero experienced chronic disease, including cancer, later in life (Petrick et al., 2009).

Chapter 2.6 – Rice and Arsenic

Rice absorbs arsenic from soil or water, thus high levels of arsenic from pesticides or herbicides can alter crop yields, and contribute to arsenic bioaccumulation with human and animal food ingestion. Brown rice showed a higher arsenic concentration in outer layers of the grain, than white rice. Polishing white rice in the processing of the grain removes surface layers, reducing the total organic and inorganic arsenic in the grain. Cereals are also higher in arsenic concentration levels. Infants who consume 2-3 servings of rice cereals per day accumulate higher concentrations of arsenic. Expectant mothers and their fetuses, as well as small children with developing brains, are at a higher risk. Rice drinks can contain up to 4.5 µg/of arsenic per drink, posing a risk for children under age 5. In utero exposures can often lead to lung cancer and bronchiectasia later in life. The best testing measurement of arsenic is a urine analysis for MMA and DMA(V) metabolites a few days after ingestion. A National Health and Nutrition Examination Survey (NHANES) study from Dartmouth University researchers shows that rice consumption contributes to increased urinary arsenic levels in children. Ingestion of seafood containing AsB may confound results. The solution to arsenic consumption in rice is phasing out the use of pesticides and fertilizers containing arsenic (Davis, et al., 2012).

Chapter 2.7 – Arsenic in Flora

Deleterious effects to humans and animals through dietary intake of the toxic metalloid and especially in marine aquatic settings are of great concern (Rahman et al., 2012). The arsenic intake by phytoplankton, the most common primary producer in marine food chains, taking up As^{5+} and reducing it to thermodynamic and unstable As^{3+} explains the ratio of As^{3+} to As^{5+} in marine waters. Algae contain high concentrations of arsenic. Brown algae contains up to 230 $\mu g/g^{-1}$; green algae up to 23.3 $\mu g/g^{-1}$, and red algae up to 39 $\mu g/g^{-1}$. Macro-algae contains 4.3-247 $\mu g/g^{-1}$ more arsenic than green and blue-green macro-algae (8.0-1.10 $\mu g/g^{-1}$ and 10.4-18.4 $\mu g/g^{-1}$, respectively) (Rahman et al., 2012).

Marine algae can hold up to 1000 times the arsenic concentration in surrounding waters, contributing to the trophic transfer of arsenic to higher levels of the marine food chain, a real threat to marine and human health. As^{5+} is taken in by phytoplankton and converted to As^{3+}. Phytoplankton plays a role in the speciation, biotransformation, and distribution of arsenic in the aquatic food chain. As^{3+} redox reactions are common in aquatic settings, with methyl conversions and organic arsenic such as arsenosugars (A_SS). Marine phytoplankton show various concentrations of arsenic, due to the biotransformation of arsenic species to methylated species in the cell of the phytoplankton. As^{5+} is taken up by the phytoplankton and incorporated into the carbohydrate compounds, and is biosynthesized to organoarsenicals. AsB increases in the marine food chain at all trophic levels, and some research considers it a non-toxic form of arsenic contained in seafood. Some research suggests that levels of AsB are elevated in crustaceans with as much as 100 µg/g/L, posing little risk to the organism or its consumer. Some research also suggests that the microflora of the crayfish degraded the AsB to TMAO, DMA(V),

methylarsenic, and an unknown substance assumed to be dimethylarsenol acetate (DMAA) (Devesa et al., 2005; Rahman et al., 2012).

Freshwater algae are lower in arsenic concentrations than marine algae. DMA(V) is converted from inorganic arsenic through biological cycling in the marine environment. As^{5+} is stable and the predominant species of arsenic in the aquatic environment transformed to As^{3+}, methylated to MMAA and DMAA by phytoplankton as well. Research suggests that the process of conversion is a detoxifying process by phytoplankton with As^{5+} and the conversion to As^{3+} then released back to the water column as DMAA and MMAA. Bacteria is also a processing factor in methylation and in mineralizing inorganic and organic arsenic (Hasegawa et al., 2001). Mineralizing bacteria plays a role in the conversion of As^{5+} to As^{3+}, DMAA and MMAA in marine waters through demethylation and oxidation of methylarsenicals. Methylarsenicals are the intermediate compounds in biosynthesis pathways of complex arsenosugars in marine phytoplankton. Marine phytoplankton reduces inorganic arsenic to Methylarsenicals through oxidative methylation from SAM to DMAA(V), and then reduced to DMAA(III). Archaea and bacteria have the ability to transform inorganic arsenic to methylarsenicals (MMAA and DMAA), to the organoarsenic species and then to arsenosugars (Rahman et al., 2012).

Chapter 2.8 – Arsenobetaine Transformation

AsB is biotransformed at ambient and at lower temperatures with microbial action through methylation by bacterial flora. The degradation pathway starts with cleavage of the carboxyl methyl-arsenic bond of AsB, which generates TMAO, with subsequent cleavage of methylarsenic bonds to form dimethylarsinate and methylarsinate (MA) (Devesa et al., 2005). The biodegradation process ends with the formation of inorganic arsenic (AB, TMAO, DMA, MA, As^{3+}, and As^{5+}). Another pathway for AsB is the cleavage of methylarsenic bonds, generating DMAA. Degradation explains the lack of AsB in marine waters. This process was executed by actively degrading bacteria strains in vitro, acting on AsB (Devesa et al., 2005). The active AB transformation to DMA, MA, and U1 (possibly MMAA) occurred in the hepatopancreas and tail, while trimethylarsonioriboside was also detected in the crayfish samples tested (Devesa et al. 2005). As^{5+} is methylated in marine waters to the less toxic MA and DMA (Contreras-Acuna et al., 2013).

Methylated trivalent arsenicals (MA(III) and DMA(III)) are more toxic than the pentavalent forms, but are still carcinogenic. Marine organisms may then transform these compounds into non-toxic species such as AsB found in seafood. AsB is the arsenic species with traces of MA(V), DMA(V), tetramethylarsonium (TETRA -- an ion), and TMAO (Contreras-Acuna et al., 2013).

Dimethylarsonioribosides and trimethylarsonioribosides are the derivatives of arsenosugars associated with the arsenic species in marine algae. In mammals, As^{5+} and As^{3+} are more toxic than the methylated forms MA(V) and DMA(V). TMAO and TETRA are moderately toxic as well. Research suggests that AsB, and Sc-arsenocholine are considered innocuous to

humans. Arsenosugars may be toxic, causing carcinogenetic effects to humans and animals (Contreras-Acuna et al., 2013).

Chapter 2.9 – Arsenic in Fertilizers, Pesticides and Herbicides

In 1867, Paris green, an arsenic-laden rodenticide and insecticide, was used on potatoes in Colorado (International Agency for Research on Cancer, 1991; Richardson, 2012). Lead arsenate and London purple, other arsenic pesticides, both followed, and over 100 million people were affected by arsenic and lead poisoning (Richardson, 2012). Recent usages fall into 2 categories: livestock drugs and pesticides. Arsenal livestock drugs like roxarsone, nitarsone, carbasone, and arsanilic acid were used in turkeys, chickens, and swine, showing up in the livers of treated animals. Roxarsone was used for weight gain and disease prevention (International Agency for Research on Cancer, 2012). Pfizer removed it from the market in 2011 (FDA, 2011). Most organic arsenals were banned in 2006, although some still remain. Outcry from industry superseded environmental pushback and prevented complete eradication of agricultural arsenic (Richardson, 2012). In 2009, all arsenals were set to be phased out by 2013, except monosodium methyl arsenate (MSMA), which was approved for usage in the cotton growing process (IARC, 2012. However, nitarsone, carbarsone, and arsanilic acid are still legal, in use, and on the market. (Richardson, 2012) Nitarsone is the last recognized arsenic drug being used in poultry farming for weight gain, but by the end of 2015, the FDA will no longer allow its use (FDA, 2015). Ironically, organic farmers cannot use arsenical pesticides, but can still use manure from chickens fed arsenals or arsenal drugs, facilitating arsenic to farmlands (Agency for Toxic Substances and Disease Registry, 2006). Most fertilizers include lime for pH adjustment with nitrogen, phosphorous, and potassium depending on soil type (Gianessi, 2009).

The EPA allows sewerage sludge containing arsenic up to 41,000 ppb to 41,000 ppm, with farmlands calculating the sludge per hectare. The sewer sludge fertilizer may also be utilized in home and garden use (Richardson, 2012).

At present, rice farms Louisiana use Round Up, a herbicide (Richardson, 2012), and malathion, an organophosphate, as an insecticide. Malathion is an endocrine disrupter that inhibits cholinesterase in insect brains, and causes death (ATSDR, 2003). Permethrin, a pyrethroid synthetic chemical used as an insecticide, acts on neurons in insect brain, affecting sodium channel activation, which also results in death of insects. Permethrin is not retained in the soil as long as most insecticides, with a biological half-life of approximately 2 weeks (Becker & Mergel, 2010). Confirm is an insecticide analogous to tebufenozide used in sugar cane. Furadan, a carbamate, is a neurotoxic pesticide that inhibits cholinesterase.

Chapter 2.10 – Arsenic Exposure

Water

Drinking Water – 10 µg/L (10 ppb) (WHO, 2001)

Soil Concentrations

Mean background concentrations – 5 mg/kg (low 1 mg/kg to high 40 mg/kg) (5,000 – 40,000 ppb) (WHO, 2001)

Mean sediment concentrations 5 – 3000 mg/kg (5,000 – 3,000,000 ppb) (WHO, 2001)

Higher levels occurring in areas of anthropogenic contamination (WHO, 2001)

Dietary Exposure

Most foods – < 0.25 mg/kg (250 ppb) (WHO, 2001)

Seafood, marine fish – 2.4 – 16.7 mg/kg (2,400 – 16,700 ppb) (WHO, 2001)

Mussels – 3.5 mg/kg (3,500 ppb) (WHO, 2001)

Crustaceans – 100 mg/kg (100,000 ppb) (WHO, 2001)

Grain – 1 – 21 µg/kg (1 – 21 ppb) (EPA)

White rice avg. – 114 µg/kg (114 ppb) (FDA)

Brown rice avg. – 59 µg/kg (59 ppb) (FDA)

Beer – 10/65 samples 15 – 26 µg/kg (15 – 26 ppb) (FDA)

Apple juice – 23 µg/kg (23 ppb) (FDA)

Dietary Intake – Ages

Infants under 1 year – 1.3 – 15.5 µg/day (1.3 – 15.5 ppb) (WHO, 2011)

2 years – 4.4 µg/day (4.4 ppb) (WHO, 2001)

25 – 30 years men – 9.9 µg/day (9.9 ppb) (WHO, 2001)

60 – 65 years women – 10 µg/day (10 ppb) (WHO, 2001)

60 – 65 years men – 13 μg/day (13 ppb) (WHO, 2001)

Standards and Guidelines – Inorganic Arsenic
Minimum Risk Levels (MRL)

Oral Acute duration – 0.005 mg/kg/day – based on LOAEL – 0.05 mg As/kg/day (5 – 50 ppb) (ATSDR, 2007)

Oral Chronic duration – 0.0003 mg/kg/day – NOAEL – 0.0008 mg As/kg/day (3 – 8 ppb) (ATSDR, 2007)

Chronic duration for skin and gastrointestinal (10 – 100 ppb) (ATSDR, 2007)

Rice – 0.2 mg/kg (200 ppb) (CAC)

Chapter 3 – Cadmium

Cadmium (Cd) is an environmental pollutant, toxic to both humans and animals, and is recognized as an occupational hazard worldwide. Industrial uses have caused great abundances of the substance, and it negatively affects human organs from acute and chronic intake. Cadmium has a biological half-life of 10-30 years, and is excreted slowly from the body while accumulating in the kidney, blood, liver, and reproductive organs. Half-life of cadmium in blood serum as compared to urine levels is a strong indicator of long-term cadmium exposure (Samuel et al., 2011).

The MRLs for cadmium are: oral: intermediate, 0.0005 mg/kg/day (0.5 ppb) and oral: chronic, 0.0001 mg/kg/day (0.1 ppb) (ATSDR, 2015). Cadmium causes nephro-toxicity, immuno-toxicity, osteo-toxicity, teratogenicity, carcinogenicity, and reproductive toxicity. Cadmium crosses the blood-brain barrier, affecting fetal development. Research suggests that gestational cadmium treatment induces ovarian toxicity and reproductive dysfunction by way of oxidative stress (Samuel et al., 2011). Pregnant and lactating females absorb and retain cadmium, transferring it to the fetus. In pregnant rats, cadmium is known to induce placental necrosis and hemorrhages, and to increase fetal deaths. Occupationally-exposed pregnant women showed lower birth weights that correlated to cord blood and maternal cadmium concentrations. Mothers of preterm infants showed a higher blood concentration of cadmium than those of full term in areas with higher soil concentrations of cadmium (Samuel et al., 2011).

Adverse pregnancy outcomes such as reduced birth size, weight, or head circumference, and preterm delivery are cadmium-related effects. Pregnant women in rural areas showed increased concentrations of cadmium in placentas. Zinc transfer in cord blood is also associated with elevated cadmium levels in placentas of pregnant women. (ATSDR, 2012) Cadmium

disturbs or inhibits zinc transport of nutrients to the fetus, causing nutritional problems associated with the placenta (Samuel et al., 2011).

When analyzing urine, cadmium levels are adjusted for specific gravity and creatinine levels (ATSDR, 2012). Smokers are not usually included in cadmium case studies, unless smoking is the epidemiological research medium in question. Urinary cadmium concentrations are better biomarkers for accumulation levels in humans than cord blood indicators, associated with inverse zinc transfers to the fetus (Samarawickrama & Webb, 1979).

Cadmium is an endocrine disrupter (Henson & Chedrese, 2004). Glucocorticoids convert maternal cortisol to cortisone. In expectant mothers, cadmium decreases levels of 11B-IISD2, while increasing cortisol levels, which may impair fetal growth. Cadmium may also interfere with IGF and the IGF axis, affecting a sex specific factor. The effect of glucocorticoids seems to be female-specific (Samarawickrama & Webb, 1979).

Cadmium effects on embryonic development and fetal gestation in rodents create a high susceptibility to adverse effects. A single dose equivalent of 40 µM of CdC_{12}/kg body weight between day 17-21 of gestation resulted in destruction of the placenta and death of embryos in a high proportion of pregnant rats (Samarawickrama & Webb, 1979). In a dose equal to 1.05 to 1.77 mg/kg administered to a Wistar-Portion strain of female rats within a 21-day gestational period, flush extremities, vasodilatation, rapid shallow respiration, apathy, and flaccidity of muscles occurred after 1,D7,0 drops were administered to gravid and non-gravid animals. Deaths occurred within 16-24 hours; with higher doses, death occurred within 6 hours. Deleterious effects were sub-pleural hemorrhage of the lung. Vaginal bleeding occurred in pregnant animals at 8 hours, indicating placenta damage. Seizures preceded respiratory paralysis and muscular flaccidity (results of neuromuscular block) (Samarawickrama & Webb, 1979).

Observations of the liver and kidneys shows excess fluid as well as an increase in blood flow. The placenta loses architecture and turns into a blood clot. The proximal tubules show complete degeneration, while glomeruli tubules are spared. Cadmium causes congenital deformities in rats, mice, and hamsters during periods of gestation. Teratogenic effects were prevalent in gestational periods of 8-15 days in rats receiving a 1.25 mg dose of cadmium, inducing deformities in a high percentage of fetuses. Encephalitis is most noticeable in 80% of fetuses after delivery by C-section. Umbilical hernias are present in 45% of the fetuses. (Samarawickrama & Webb, 1979).

Cadmium distribution to the embryo on day 12 of gestation interferes with the placental function, and the zinc transport mechanism demand to the placenta. A dose of 0.5 mg/kg after four hours produces inhibition of zinc across the placenta by as much 50%, and the developing brain is also affected. Thymidine incorporation into the embryo's DNA is also inhibited at 4-20 hours, but is similar to controls in the four hour interval. DNA synthesis is inhibited; RNA and protein production are unaffected (Samarawickrama & Webb, 1979).

Maternal cadmium retention from oral exposure during gestation is double the concentration observed in non-pregnant mice, but a small fraction of cadmium is still transferred to the fetus in exposed mice. Results show that chronic cadmium exposure causes behavioral changes in mice offspring. Neuro-chemical parameters are being explored to the actual effects of cadmium on growing pups, with long-term effects on brain functions during childhood (Gupta, et al., 1991).

Assays were performed with acetyl cholinesterase activity in the homogenate, using acetyl thiocholine iodide substrate in female albino rats of the Durkey strain. The acetyl cholinesterase enzyme was increased by 13% in cadmium-exposed pups at ages 14 and 21 days

compared to control rats. The sodium-potassium pump production in rat controls increased significantly (20%) from age 7 to 21 days. The enzyme 2', 3'-cyclic nucleocide 3'-phosphohydrolase (a myelin protein) showed an increase from 7 to 21 days in control animals, as compared to and the same at postnatal 7 days, but lower by 29% and 24% at 14 and 21 days, as compared to the control. The 5'-nucleotidase (enzyme that catalyzes RNA reactions) was identical in control 7 to 14 day-old pups, increased in 21 day-old controls, but was 21% lower in experimental pups. Overall, activity of this enzyme was lower in gestational-exposed pups (Samuel et al., 2011).

Cadmium causes changes in production of ovums and in regulation of hormones secreted by the pituitary and hypothalamus glands. It also causes a decrease in body and ovary weight in cadmium-treated animals. Impaired gonadal development causes gonadotropin levels to decrease. Steroid hormone production is also impaired, decreasing testosterone, estradiol, and progesterone in cadmium treated rats (Samuel et al., 2011).

Cadmium inhibits germ cell production in developing human female and male gonads. Research suggests that a 1 µM concentration (a very low exposure) decreases human germ cell development, showing extreme sensitivity of germ cells to cadmium exposure. In other human cells like hepatocytes and neuroblastomal cells, a 100 µM concentration triggers deleterious effects. One µM of cadmium decreases germ cell counts after 3 days of exposure through apoptosis, but does not alter germ cell proliferation. Cadmium alters DNA repair in proliferating germ cells, causing deleterious effects to meiotic cells undergoing apoptosis. Oocytes in mice exposed to cadmium decreased due to apoptosis (Angenard et al., 2010).

Brain enzymes increase from birth to adulthood due to cellular development fractions, reflecting the increased functional brain activity. Cadmium inhibits a wide variety of

mitochondrial functions, including SDH-sorbitol dehydrogenase. In 21-day-old pups, enzymes were inhibited by cadmium. Research indicates that lower level exposure to cadmium causes behavioral problems at 60 days. In utero exposure to cadmium causes changes of neurochemicals in the brain, responsible for functional developmental changes (Gupta et al., 1991).

Pancreatic cancer is another side effect of cadmium exposure in humans. South Louisiana has experienced a high mortality rate from pancreatic cancer since 1950. Information obtained from Region 4 Tumor Registry of Louisiana, provides demographic information (Luckett et al., 2012). The Acadiana region from 2000-2004 had the highest incidence rate of cancer with 15.9 cases per 100,000 residents. Cadmium causes trans-differentiation of pancreatic cells and increases synthesis of DNA in the pancreas. Cadmium oxide is released during cigarette burning, showing 3-4 times higher accumulation levels in smokers versus non-smokers; cigarette smoking is an established risk factor of pancreatic cancer, providing 1 to 2 micrograms in each cigarette (Luckett et al., 2012).

Phosphate fertilizers and sewerage sludge contains cadmium, while cadmium chloride is used in the preparation of some pesticides, raising cadmium levels in soil. Household dust testing in South Louisiana in rural and urban areas showed higher mean-rates of cadmium levels. Cadmium is released in gasoline and lubricating oils during fossil fuel combustion. Welders and pipe fitters are subjected to fumes generated in shipyard construction. Research suggests, cancer is prevalent in South Louisiana especially from cereal and rice products, accounting for 66% from plants and 33% from animal sources (Luckett et al., 2012).

Consumption of red meat and pork are both associated with a higher risk of cancer, due to cadmium intake. Research suggests that grain feed, especially that fed to pork, may have a higher cadmium level. Red meat is a product of grass grazing, indicating less cadmium intake.

Cadmium exposure beyond threshold limits is transmitted from females to fetuses, causing DNA inhibition and increasing percentages of cancer. Fetal gestation is often times the beginning of cadmium accumulation, often becoming cancerous after age 40. The body has no efficient excretion method for removal of the heavy metal, thus it accumulates in utero to adulthood in tissues and organs. Cadmium binds to metallothionein (MT) in low levels and is usually deposited in the pancreas, liver, kidneys, and testes (ATSDR, 2012). Cadmium competes with zinc in biochemical reactions. Sharing many physical and chemical properties, biological systems that require zinc are often replaced by cadmium, introducing the formation of tumors (Schwartz & Reis, 2000).

Dietary cadmium is associated with carcinogenesis and cancer-related illnesses. In western countries, vegetables and cereals contain higher sources of cadmium. Far east countries like Japan show a higher intake in rice and rice products. Low levels of cadmium have a direct effect on cancer related illnesses in the general populations. As much as 80% of cadmium intake is 8 to 25 µg/day from food sources. Gastronomical intake is lower at approximately 5 % (Cho et al., 2013).

Intake is relative to lifestyle, dietary habits, and absorption rate. Populations in certain geographical locations are more prone to exposure, due to higher concentrations. Louisiana has a higher intake of cadmium from a diet containing greater proportions of rice, pork, and beef from grain fed animals. Higher exposure creates much greater intakes for cancer related illnesses in Louisiana. Recent studies show biomarkers related to cadmium risks and cancer illnesses. Studies show higher rates of urinary cadmium, correlating to breast cancer in breast tumor tissue (Cho et al., 2013). Limiting rice and vegetable products as well as pork and red meat in areas with higher levels of cadmium contamination will limit intake. Educating populations within

demographics subject to higher levels of cadmium will help control cancer caused by the heavy metal (Luckett et al., 2012)

Cadmium is known to cause cancer by other pathways such as aberrant gene expression, oxidative stress, inhibition of DNA damage repair, and inhibition of apoptosis (Xu et al., 1996). Other studies have shown that cadmium mimics steroid hormones as well as androgen and estrogen binding, while activating steroid receptors (Johnson et al., 2003). Research suggests that cadmium may facilitate other carcinogenic compounds to induce cancer as well, although carcinogenesis can be caused solely from cadmium exposure (Cho et al., 2013).

Smokers have a higher cadmium level in the lung than non-smokers. The lung is a major target organ, accumulating and absorbing cadmium with a nine-and-a-half-year half-life. Occupational cadmium affects approximately 500,000 workers in the US. Long-term exposure to cadmium causes emphysema, by cadmium binding to thiols, MT, and glutathione. Emphysema by definition is "a pathological lesion of abnormal enlargement of the respiratory airspaces, very common in industrial workers chronically exposed to cadmium" (Zhao et al., 2006). Chromosomal aberrations increased in the lymphocytes of cadmium exposed workers (ATSDR, 2012). After attaining thresh-hold levels, cancer causing effects are endocrine interruption, DNA synthesis, and aberrant gene expression.

Cadmium (2^+) and zinc (2^+) ions have the same electronic structure of the valence shell. Cadmium acts as a zinc antagonist. Both compete for the metal-binding protein MT. Cadmium has a greater affinity and binds to MT, reducing distribution of the metal to the tissues (other than the liver, where it does accumulate) and promotes elimination by way of synthesis. It causes renal toxicity. Free cadmium is toxic; bound cadmium is nontoxic (Valko et al., 2005; ATSDR, 2012).

Cadmium is absorbed by blood plasma, binding to albumin and other high weight proteins. The liver takes it up and induces MT with sulfhydryl groups, binding cadmium efficiently with a small amount entering blood circulation. Cadmium-metallothionein is filtered in plasma through the renal glomeruli and reabsorbed in proximal tubules thus accumulating in the kidney. Fourteen days to 1 year of chronic exposure creates a major burden in the kidney. Lysosomes of the tubule cells break down the cadmium-metallothionein complex; free cadmium induces renal synthesis of MT, binds to cadmium and decreases in toxicity. When synthesis capacity of MT with cadmium is exceeded, free cadmium accumulation increases. Some cadmium from the kidneys is continuously released into the blood stream, crossing the blood-brain barrier with smaller proportions and distributing to other organs, such as the pancreas and testes (Luckett et al., 2012).

Cadmium disrupts normal endocrine function by inducing oxidative stress in the reproductive organs such as the ovaries and testes (Thompson & Bannigan, 2008; Siu et al., 2009). Mammalian studies show reduced sperm count as well as poor semen quality in men exposed to cadmium (Benoff et al., 2000; Siu et al., 2009).

The main organs affected by high-dose, acute exposure of cadmium with chronic effects are the kidneys, bones, and especially the lungs. Cadmium is primarily excreted in the urine, with some released through bile, pancreatic juice, and feces. Elimination of cadmium from the kidney is very slow, with the highest level of cadmium concentration in the renal cortex (ATSDR, 2012).

Cadmium catalytically increases oxidative stress in the formation of the reactive oxygen species (ROS), also increasing lipid peroxidation, depleting glutathione, and protein-bound

sulfhydryl groups, while eliminating the protective junction of nitric oxide formation (Valko et al., 2005). Cadmium also stimulates the production of inflammatory cytokines.

DNA strand breaks cause mutations aligned with chromosomal damage, cell transformation, and impaired ability to self-repair (Beyersmann & Hartwig, 2008). Cadmium has been found to cause chromosomal damage in animal experiments with subcutaneous administration. Cadmium causes cancer of the lung, kidney and prostate. (ATSDR, 2012)

Blood levels of cadmium are monitored for exposure. Blood levels decrease after exposure and a slow elimination commences, eliminating cadmium from the kidney and body. Cadmium is measured in whole blood erythrocyte, serum bound levels, and metallothionein bound cadmium in plasma. (ATSDR, 2012)

Urine is used as a primary indicator of exposure. A 24-hour specimen is used to show the relationship to and variation between the cadmium count in urine and the kidney. A substantially high cadmium content in the renal cortex is enough to cause tubular damage. Due to cadmium content in smokers, there is a variation between non-smokers below 1µg/g creative (-1µg/I or µg/24 hr) (ATSDR, 2012). Other means of determining cadmium levels in the liver and kidney are the neutron activation analysis and x-ray florescence. These can detect the damage due to long-term exposure (ATSDR, 2012).

Cadmium overexposure produces skeletal effects, with excess excretion of calcium causing bone pain, osteomalacia and osteoporosis (Luckett et al., 2012). Bone effects were first recognized during WWII in Japan with renal dysfunction and osteodystrophy in postmenopausal, multiparous women. They were living downstream from an old lead and zinc mine, and drinking from a contaminated water supply. Osteomalacia (referred to in Japan as “Itai-itai disease” or “Ouch-Ouch disease”) painfully manifests in the bones (Yoshida, 1999). Pregnancy, lactation,

and menopausal hormone depletion caused by bone changes may have exacerbated cadmium osteotoxcity (ATSDR, 2012). In osteomalacia, cadmium is exchanged for calcium in the bones, and decreases bone formation. This leads to osteoporotic bone loss and increased bone fragility at low bone cadmium concentrations, and marked osteopenia as well as decreased bone density (Yoshida, 1999; ATSDR, 2012). Bone damage from a vitamin D inhibition or metabolism deficiency due to cadmium may occur hours after exposure also causing parathyroid hormone (PTH) and vitamin disturbances. Serum parathyroid hormone serum increases as well (ATSDR, 2012).

Chapter 4 – Copper

The recommended daily allowance (RDA) for copper is 0.9 mg, but the median intake from a typical U.S. diet ranges from 1-1.6 mg/day. The safest highest intake of copper for chronic exposure is 10 mg/day. Copper is found in shellfish, organ meats, nuts, beans, and cocoa (New Hampshire Department of Environmental Services, 2013). Copper is present in all living cells and is associated with many oxidation processes. Through oxidation, copper exists in one of two ionic states: cuprous (Cu) and cupric (Cu^2), which is more abundant. Copper binds to proteins such as ceruloplasmin, albumin, and transcuprein, which are all copper transport vehicles, and to the blood-clotting factor V. MT sequesters and stores the metal in cells (Linder et al., 1998).

Copper is absorbed through the duodenum and the stomach. Half of copper absorbed is taken in through the intestinal mucosa then carried by albumin to the liver. Ceruloplasmin, albumin, and transcuprein are complexed with copper and transported to tissues. Ascorbic acid inhibits the absorption of copper. Excretion occurs through bile, sweat, urine, and saliva (Wapnir, 1998).

Blood levels of copper rise rapidly after ingestion, bound to albumin, then released to other carriers for cell specific uptake. Transcuprein transports copper to the liver and kidneys, and reemerges into the plasma, now bound to the ceruloplasmin. 60-95% of copper is found bound to ceruloplasmin (Wapnir, 1998).

Ceruloplasmin does not enter the cell, but copper does via a carrier-mediated process. Adenosine triphosphatase (Cu-ATP) is a binds copper and transports it in and out of cells. Liver is a storage site and route for excretion in the bile system (Dijkastra et al., 1995).

Ceruloplasmin is synthesized in the liver. Indicators of copper exposure are detected in serum and urine, as well as ceruloplasmin levels. Blood proteins and MT bind copper, which is an essential component of metalloenzymes. Impairment of metalloenzymes can cause anemia, infections, bone abnormalities, hypopigmentation of hair, and hypotonia (Dijkastra et al., 1995).

Copper toxicity results in gastrointestinal symptoms, hepatic necrosis, and even death. Wilson's disease (a rare inherited genetic disorder) is caused by excessive accumulation of copper in the liver, brain, kidney, and cornea (Ferenci et al., 2015). Accumulation in the liver results in impaired biliary excretion of copper. It is common in Hungary, Yugoslavia, and Italy (especially in Sicily) (Loudianos et al., 2003; Adhami & Cullufi, 1995). Brass vessels for feeding and drinking in children can nurture jaundice, liver disease, fibrosis, and cirrhosis from copper contamination; in western countries idiopathic copper toxicosis occurs from milk stored in copper vessels (Loudianos et al., 2003; Adhami and Cullufi, 1995).

In children, symptoms show up by age 4 in abnormal posture or stiffness of legs and arms. Other symptoms are confusion, delirium, dementia, ataxia (impairment of muscle coordination), emotional changes, and abdominal distension (Loudianos et al., 2003). Sufferers of Wilson's disease also experience personality changes, phobias, neuroses, slower facial expressions, arm tremors, uncontrollable or jerky movements, vomiting blood, jaundice, yellow eye color, and overall weakness (Ferenci et al., 2015).

Copper is associated with Menkes disease, a sex-linked inherited characteristic, primarily affecting male infants. It is referred to as “Pili torti,” "steely haired disease" or "kinky hair syndrome" where the hair is kinky, lacking color, or steel colored, and very easily

broken hair shafts (Choudhary et al., 2012). There is distinct abnormality in the hair of males, whereas in females only half of hair follicles will be noticeably abnormal, but readily noticeable under a microscope (Shapira et al., 1992). In Menkes disease, mutation of the ATP7A gene inhibits the distribution of copper to body cells. Copper accumulates in tissues such as the small intestines and kidneys (Choudhary et al., 2012). The brain usually has low levels of copper. A decrease in the supply inhibits the usefulness of many copper-containing enzymes necessary for the structure and function of bone, skin, hair, and blood vessels, as well as the central nervous system. Signs of Menkes disease and occipital horn syndrome are caused by reduced activity of these copper-containing enzymes. Lack of muscle control (hypotonia), seizures, degeneration of the cerebral cortex, and mental degeneration sometimes yields death by age 5 (Choudhary et al., 2012; Tang et al., 2006).

High or high normal zinc and low ceruloplasmin levels can cause spastic gait, dysfunction and polyneuropathy. Normal or near resoration of copper levels can be achieved with supplements and prevention of neurologic deterioration, which can be caused by prior gastric surgery, excessive zinc ingestion, and malabsorption. Likened to zinc deficiency, hematological effects of copper deficiency can inhibit incorporation of iron into heme molecules (Wapnir, 1998).

Copper is essential in certain redox reactions, and deficiency can cause toxicity and impairment of the body's oxidative system. This will inhibit the antioxidant defense system of cells, possibly causing cardiovascular rupture, weakening of cross-linking, fibrosis of the cardiac muscle, heart atrophy, aortic syndrome-aneurysm, and increased risk of disruption of normal cardiac rhythm, as well as hemopoietic, vascular, skeletal, and CNS damage. (Kumar, 2006)

Chapter 4.1 – Menkes Disease

Menkes disease is indicative of neurological defects in a group of enzymes requiring copper. Menkes disease was first described in a single family in 1962. The rate of occurrence is 1/140,000 to 1/300,000 with known neurogenic defects. Idiopathic occurrences are poorly comprehended. Asian research has calculated the occurrence at 1/50,000 to 1/300,000 of live births, affecting males more often than females (Lin et al., 2014; Kaler, 2011). Research has found that mutations in the ATP7A gene causes a loss of copper in the body's cells (Zlatic et al., 2015). Pathogenic mechanisms include neuronal pathogenesis of copper from a micronutrient to a toxicant, causing cell death triggered by altered metabolic homeostasis mechanisms and cells using neurotoxic anticancer agents, such as platinum, to bind to ATP7A (Zlatic et al., 2015).

Alzheimer's disease and Parkinson's disease are further complications of Menkes disease due to impairment in molecular networks. Menkes disease causes hypotonia, failure to thrive, focal and generalized seizures, impaired cognitive development, and brain atrophy. Menkes disease also causes sagging infant facial appearances, arched palates, laxity of skin, reduced bone density, bladder diverticula, and aneurysms. Gray and white brain matter atrophy is another complication: neuronal cell loss in the cerebral cortex, hippocampus, striatum, hypothalamus, and thalamus can occur. Astrocytosis is indicative of cell loss in the cerebral cortex (Zlatic et al., 2015).

ATP7A copper extrusion supplies the body with copper transport across intestinal enterocytes, accounting for severe systemic copper deficiency in Menkes patients (Zlatic et al., 2015). Color exemplification and hypo-pigmentation is also observed (Zlatic et al., 2015). Research suggests that early diagnosis and copper supplements can improve neurological

problems associated with the disease, but not the physiological. No effective cure has been reported to date (Lin et al., 2014; Kaler, 2011).

Chapter 4.2 – Wilson's Disease

Wilson's disease occurs usually in the second or third decade of life, with 46 of 122 patients (3.8%) displaying symptoms after 40 years of age. Cellular level indicators of pathogenic ATP7B amino acid substitutions may vary. Wilson's disease has also been reported in as early as 9 months of age. Pediatric physicians should be aware of spasmodic muscle cramps and myopathy in children. Symptoms of wing-beating, tremor, or flapping tremor combined with dysarthria, suggest the diagnosis of Wilson's disease. The most symptomatic indicator is a dystonic tremor. Aysarthria is combined with slow tongue movements, orofacial dyskinesias, involuntary grimacing with open mouth, and contracted upper lip (Bandmann et al., 2015). Increased copper intracellular concentrations can create oxidative stress, free radical formations, and mitochondrial dysfunction. These combined effects can lead to cell death in hepatic and brain tissue, and in other organs (Bandmann et al., 2015). A large statistical study in Asia revealed the prevalence of Wilson's disease; a ratio of 1/1500 – 1/3000 based on concentrations in serum of the copper-carrying protein ceruloplasmin was established (Bandmann et al., 2015).

Research suggests that three distinct neurological disorders are usually present in Wilson's disease: dystonic syndrome, ataxic syndrome, or a Parkinsonian syndrome with most patients presenting a combination of symptoms. Other movement impairments are hypomimia, shuffling gait, impaired fine finger movements, and foot tapping (Bandmann et al., 2015).

Analysis of urine in a 24-hour interval to determine copper excretion is an important diagnostic test for Wilson's disease. In asymptomatic children, 40 µg/24 hr (0.64 µM/24 hr) are suggestive of Wilson's disease. Two mutations associated with ATP7B in Wilson's disease can be detected and confirmed clinically if the entire coding region and adjacent splice sites are sequenced. This process can expose and analyze the disease in families with a higher propensity

and prevalence of the disease. Treatment of the disease includes two phases of chelation and de-coppering therapy, or botulinum toxin injections or primidone for dystonia (Bandmann et al., 2015).

Chapter 4.3 – Copper Exposure

Water

Drinking water – 1.3 mg/L (1,300 ppb) (ATSDR)

Bottle water – 1.0 mg/L (1,000 ppb) (ATSDR)

Dietary Intake – Ages

Recommended Daily Allowance

Infants 0 – 6 months – 200 µg/day (200 ppb) (NA, 2001)

Infants 7 – 12 months – 220 µg/day (220 ppb) (NA, 2001)

1 – 3 years – 340 µg/day (340 ppb) (NA, 2001)

4 – 8 years – 440 µg/day (440 ppb) (NA, 2001)

9 – 13 years – 700 µg/day (700 ppb) (NA, 2001)

14 – 18 years – 890 µg/day (890 ppb) (NA, 2001)

≥ 19 years – 900 µg/day (900 ppb) (NA, 2001)

Pregnancy ≤ 18 years – 1,000 µg/day (1,000 ppb) (NA, 2001)

Pregnancy 19 – 50 years– 1,000 µg/day (1,000 ppb) (NA, 2001)

Lactation ≤ 18 years – 1,300 µg/day (1,300 ppb) (NA, 2001)

Lactation 19 – 50 years – 1,300 µg/day (1,300 ppb) (NA, 2001)

Estimated Average Requirements

1 – 3 years – 260 µg/day (260 ppb) (NA, 2001)

4 – 8 years – 340 µg/day (340 ppb) (NA, 2001)

9 – 13 years – 540 µg/day (540 ppb) (NA, 2001)

14 – 18 years – 685 µg/day (685 ppb) (NA, 2001)

≥ 19 years – 700 µg/day (700 ppb) (NA, 2001)

Pregnancy 14 – 18 years – 785 µg/day (785 ppb) (NA, 2001)

Pregnancy 19 – 50 years – 800 µg/day (800 ppb) (NA, 2001)

Lactation 14 – 18 years – 985 µg/day (985 ppb) (NA, 2001)

Lactation 19 – 50 years – 1,000 µg/day (1,000 ppb) (NA, 2001)

Tolerable Upper Intake Levels

Infants 0 – 6 months – ND* (NA, 2001)

Infants 6 – 12 months – ND* (NA, 2001)

1 – 3 years – 1,000 µg/day (1,000 ppb) (NA, 2001)

4 – 8 years – 3,000 µg/day (3,000 ppb) (NA, 2001)

9 – 13 years – 5,000 µg/day (5,000 ppb) (NA, 2001)

14 – 18 years – 8,000 µg/day (8,000 ppb) (NA, 2001)

≥ 19 years – 10,000 µg/day (10,000 ppb) (NA, 2001)

Pregnancy 14 – 18 years – 8,000 µg/day (8,000 ppb) (NA, 2001)

Pregnancy 19 – 50 years – 10,000 µg/day (1,000 ppb) (NA, 2001)

Lactation 14 – 18 years – 8,000 µg/day (8,000 ppb) (NA, 2001)

Lactation 19 – 50 years – 10,000 µg/day (10,000 ppb) (NA, 2001)

* ND = Not determinable due to lack of data of adverse effects in this age group and concern with regard to lack of ability to handle excess amounts. Source of intake should be from food only to prevent high levels of intake.

Standards and Guidelines
Minimum Risk Levels (MRL)

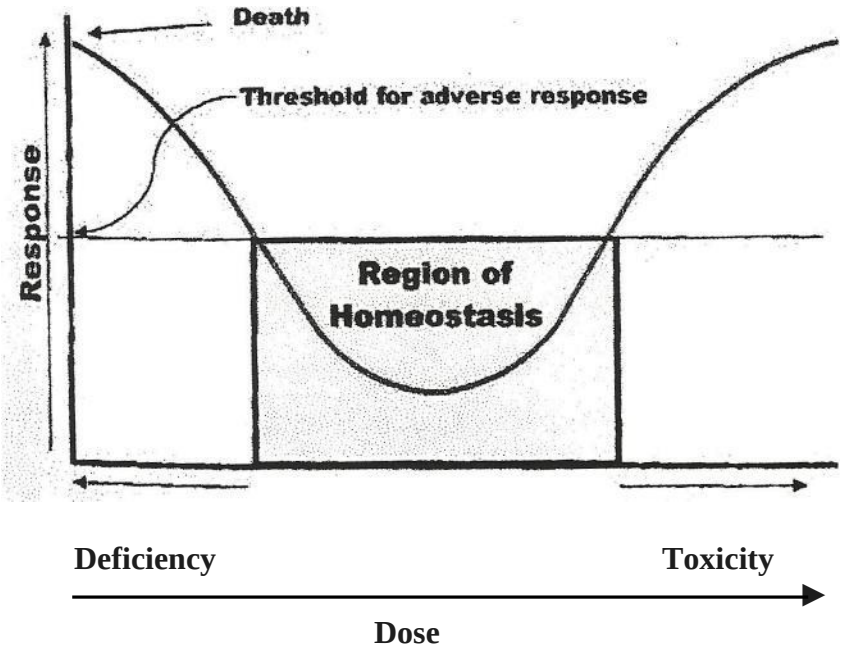
Oral Acute duration – 0.01 mg/kg/day gastrointestinal (10 ppb) (ATSDR)

Oral intermediate duration – 0.01 mg/kg/day gastrointestinal (10 ppb) (ATSDR)

Chapter 5 – Zinc

Zinc and Copper are nutrients required for normal physiological function in the human body. The bottom of the U-shaped curve in Figure 1 represents the region of homeostasis -- the dose range results in neither deficiency nor toxicity.

Figure 1 – Homeostasis



(Calabrese & Baldwin, 2001)

Genetic expression, cell division, protein synthesis, immune functions, wound healing, and normal growth are all essential for the function of greater than two hundred enzymes. Activity and structure require zinc for carbonic anhydrase, alkaline phosphate, alcohol dehydrogenase, copper-zinc superoxide dismutase, carboxypeptidase, RNA polymerase, and reverse transcriptase (Calabrese & Prasad, 1983; Nriagu, 2007).

Zinc inhibits copper absorption through MT in the genetic treatment of Wilson's disease in which copper accumulates in cells. Absorbed copper is bound by MT in mucosal cells,

preventing its transport into the body. Copper displaces zinc from MT and caused copper deficiency (Avan & Hoogenraad, 2015).

Zinc is found in erythrocytes (87% carbonic anhydrase, a major binding site) in plasma with two-thirds of zinc bound to albumin in the diffusible form, and the remainder bound to alpha2-macroglobulin (non-diffusible). Binding sites for zinc are limited in number, with these two blood proteins regulating the amount of zinc retained in the body -- crucial for immune function. Zinc is lost from the body through hair loss, saliva, sweat, urine and feces (ATSDR, 2003; Nriagu, 2007). MT production increases in response to increased zinc levels, regulating it by binding to the metal to aid in excretion through the feces (ATSDR, 2005).

The Food and Nutrition Board (FNB) at the Institute of Medicine of the National Academies developed dietary reference intakes (DRI). The RDA for zinc is 15 mg/day. Adequate intake (AI) is established when there is insufficient evidence to develop RDA; a level is assumed to ensure nutritional adequacy. 40 mg/day is the tolerable upper intake level (UL) (Fosmire, 1990; Nriagu, 2007).

Research suggests that zinc supplements aid in weight gain and growth factors in children (Brown, 1998). Lack of zinc supplementation can create adverse effects on fetal brain development and function in children (Merialdi et al., 1999). However, zinc supplements can cause long-term effects when taken 1-8 years. 2 mg/kg/day of zinc sulfate for 10 months caused anemia in humans. 50 mg/day of zinc gluconate for 10 weeks caused a reduction (47%) in erythrocyte superoxide dismutase activity in hematocrit and serum ferritin. Zinc induces copper deficiency and severe hematologic effects, including anemia. Ringed sideroblasts from iron deposits occur in erythrocyte precursors, and a decrease in ceruloplasmin. A study of healthy men given a dose of 200 mg of zinc for 6 weeks showed

a reduction in lymphocyte stimulation response. The study also showed reduction response to phytohemagglutinin, chemotaxis, and phagocytosis of bacteria by segmented neutrophils. High levels of zinc (300 mg or higher) causes anemia by decreasing high-density-lipoproteins (HDL – "good" cholesterol), and pancreatic damage. It also increases lower-density-lipoproteins (LDL – "bad" cholesterol), although total cholesterol was unaffected from baseline levels (Fosmire, 1990; Nriagu, 2007). Acute pancreatitis followed the ingestion of liquid zinc chloride (Nriagu, 2007).

Signs of zinc toxicity include vomiting, diarrhea, lack of appetite, abdominal pain, headaches, and lethargy. Other deleterious effects include pale gums, acute kidney failure, loss of libido, impotence, prostatitis, ovarian cysts, menstrual problems, and muscle spasms (Fosmire, 1990; Nriagu, 2007).

Zinc toxicity causes gastrointestinal distress from beverages stored in galvanized cans. Inhalation of zinc fumes causes metal fume fever (MFF), which manifests as chills, profuse sweating, and weakness, lasting approximately 24 hours. This is very common in workers on Mondays after holidays with consumption of beverages affected by zinc containers. MFF is also common with welders who work with galvanized zinc metal structures (Wardhana & Datau, 2014). The North American diet contains approximately 8-15 mg/day from data obtained by way of the 1988-1994 National Health and Nutrition Examination Survey (IOM, 2001). Zinc is absorbed primarily in the small intestine, with the duodenum absorbing approximately 60%, the ileum 30%, the jejunum 8%, and 3 % by way of the colon and cecum (Methfessel & Spencer, 1973; Davies, 1980).

Animal studies conducted on high proportionate amounts of zinc exposure in the 2000 ppm range showed epiphyseal inhibitions in the long bones of the hind legs of

laboratory rats. The study also showed paralysis of the hind legs, curvature of the lower leg, retarded epiphyseal closure of the proximal end of the tibia, proliferation of epiphyseal cartilaginous tissue, and irregular arrangement of chondrocytes (Halliasos et al., 2007; Cui et al. 2004; Lelie et al., 2011).

Diseases such as amyotrophic lateral sclerosis (ALS), a progressive neurodegenerative disease, results in a loss of upper and lower motor neurons, and eventually fatal paralysis. Studies showed that Zn^{2+} induced changes in neurotrophins, or proteins that regulate development, maintenance, and function of neurons. Alzheimer's disease studies show higher levels of zinc in the olfactory region, hippocampus, and amygdala, causing neuron death. Zinc also induces accumulation of toxic free radicals that leads to neuronal necrosis. A research study of 11 cases of multiple sclerosis in workers with 10 years of exposure in a manufacturing plant showed higher serum levels than control groups (Halliasos et al., 2007; Lelie et al., 2011).

Fixodent and Poligrip (adhesive pastes for dentures) were analyzed for zinc concentrations. A high level of 17 – 34,000 µg/gm zinc was detected. The patient used two tubes of denture cream per week for 2 years. The result of over exposure to zinc included numbness and weakness of arms and legs, often leading to wheel chair dependency (Nations, 2008). Another patient in the study wore dentures for many years, using 3 tubes a week. The patient experienced hand numbness, impaired balance, severe distal upper extremity weakness, atrophy, hyper-flexia, abnormal extensor plantar responses, and decreased pinprick sensations in the hands. A brain MRI showed sub-cortical lesions (Nations, 2008).

Zinc deficiency causes severe health effects and is a co-factor of 200 metallonenzymes that are required in immune function and for vitamin A metabolism. Zinc

deficiencies also cause delayed sexual maturation, pellagra, iron folate deficiency, ulcerative colitis, malabsorption syndromes, and chronic renal disease disorder. Other effects are growth retardation, loss of appetite, hair loss, impotence, hypogonadism, eye and skin lesions, weight loss, delayed wound healing, taste abnormalities, and mental lethargy (Halliasos et al., 2007; Nriagu, 2007). Newborns manifest zinc deficiency in hair loss, impaired speech, increased infections, and neuropsychological problems (Halliasos et al., 2007).

Chapter 5.1 – Metal Fume Fever Associated with Galvanized Welding

MFF (a flu-like illness) associated with galvanized welding is usually due to inhalation of fumes generated from molten galvanized bronze or steel welding. An estimated 1500 – 2000 cases of MFF occur each year in the United States. In the welding process, zinc is melted, producing zinc oxide fumes with particulates ranging from 0.1-1.0 μM in diameter, but aggregation can form larger particle sizes (Wardhana & Datau, 2014).

As part of the inflammatory response to zinc fume inhalation, leukocytes form from cytokine release in the lungs. Reactive oxygen species (ROS) increases, causing oxidative stress (damage to cell structures) in the bronchiolar and alveolar regions of the lungs, generating more free radical activity. Fever, coughing, wheezing, chest tightness, fatigue, chills, myalgia, cough, dyspnea, leukocytosis with a left shift, thirst, metallic taste, and salivation are symptoms of MFF. No specific treatment is advised for MFF. Flu-like symptoms may develop approximately 3 hours after exposure, with a high occurrence of respiratory symptoms or bronchial responsiveness (Wardhana & Datau, 2014).

Pathological respiratory problems may arise as well. Peripheral broncho-alveolar lavage (BAL) may be performed to determine if severe acute inflammation is present, via cell count of polymorphonuclear (PMN) leukocytes. A hypothesis of pathogenesis is the release of endogenous pyrogens from the leukocytes, as well as production of metal proteinases, or a group of enzymes that can hydrolyze proteins. The resulting metal proteinases are composed of inhaled particles and damaged pulmonary tissue, or antigens leading to the formation of allergen-antibody complexes. These complexes cause the clinical symptoms although the mechanisms are unclear for the symptoms. (Wardhana and Datau, 2014)

ROS production also increases. Oxidative stress is caused from the deposit of zinc oxide and oxidative stress, a pathway to ROS inducing a greater inflammatory response. (Wardhana and Datau, 2014)

Eighteen hours after exposure, MFF symptoms peak, and are resolved within 1-2 days. Typical treatments are anti-pyretics, analgesics, and oxygen therapy for hypoxia. Wearing masks to prevent inhalation of fumes and particulates, and plenty of ventilation in work areas will help prevent new cases of MFF (Wardhana & Datau, 2014).

Chapter *5.2 – Diseases Related to Zinc Deficiencies*

Zinc deficiency affects the epidermal, gastrointestinal, central, nervous, immune, skeletal, and reproductive systems (Hambridge, 2000). Wilson's disease and Alzheimer's disease are both age-related free copper toxicoses. Wilson's disease pathology shows free plasma copper is toxic, generating oxidative stress, free radicals, and degeneration of cortical neurons (Hoogenraad, 2011). Increases in free plasma zinc levels create MT expression and neuron damage, inflammation, and oxidative stress. Copper toxicosis in age-related Alzheimer's disease may be ameliorated by zinc therapy (Avan & Hoogenraad, 2015).

Chelation therapy is not recommended for treatment of copper toxicosis and is deemed to be dangerous (Hoogenraad, 2011). More research is needed to determine the benefits of zinc supplements. Copper toxicosis therapy can inhibit zinc supplementation to the body within homeostasis (Avan & Hoogenraad, 2015).

Zinc is present in hair, skin, nails, the immune system, metalloenzymes, alkaline phosphatase, RNA, and DNA polymerase. Zinc deficiencies are noticeable in patients exhibiting alopecia, perlèche, intractable vomiting, diarrhea, loss of appetite, apathy, and irritability. The cutaneous signs of zinc deficiencies are lesions on the erythematous base crusting over, creating a sharply marginated and lichenified form. Immune function is altered, permitting the growth of yeast and bacteria thus creating impetigo, and paronychial infections (Haliasos et al., 2007). Zinc metabolism attributes to phenotypic expression of the rare autosomal inherited disorder, acrodermatitis enteropathica, characterized by dermatitis and alopecia (Moynahan, 1974; (Hambridge, 2000).

Diarrhea may cause zinc deficiency, as it is excreted from the body due in feces. Zinc supplements in world meta-analysis of diarrheal incidences reduces the severity of loss of fluid and zinc (Brown, 1998).

Chapter 5.3 – Zinc Exposure

Water

Drinking water – 0.003 mg/L – 2.0 mg/L (2,000 ppb) (ATSDR, 2005)

Surface water – 0.002 mg/L – 1.2 mg/L (1,200 ppb) (ATSDR, 2005)

Soil concentrations

Mean – 60 mg/kg (60,000 ppb) (ATSDR, 2005)

Soil and sediment – < 5 mg/kg – 2,900 mg/kg (< 5,000 – 2,900,000 ppb) (ATSDR, 2005)

Dietary Intake – Ages

Recommended Daily Allowance

Infants 0 – 6 months – 2 mg/day (2,000 ppb) (NA, 2001)

Infants 7 – 12 months – 3 mg/day (3,000 ppb) (NA, 2001)

1 – 3 years – 3 mg/day (3,000 ppb) (NA, 2001)

4 – 8 years – 5 mg/day (5,000 ppb) (NA, 2001)

9 – 13 years males– 8 mg/day (8,000 ppb) (NA, 2001)

14 – 18 years males– 11 mg/day (11,000 ppb) (NA, 2001)

≥ 19 years males – 11 mg/day (11,000 ppb) (NA, 2001)

9 – 13 years females– 8 mg/day (8,000 ppb) (NA, 2001)

14 – 18 years females– 9 mg/day (9,000 ppb) (NA, 2001)

≥ 19 years females – 8 mg/day (8,000 ppb) (NA, 2001)

Pregnancy ≤ 18 – 12 mg/day (12,000 ppb) (NA, 2001)

Pregnancy 19 – 50 – 11 mg/day (11,000 ppb) (NA, 2001)

Lactation ≤ 18 – 13 mg/day (13,000 ppb) (NA, 2001)

Lactation 19 – 50 – 12 mg/day (12,000 ppb) (NA, 2001)

Estimated Average Requirements

Infants 6 – 12 months – 2.5 mg/day (2,500 ppb) (NA, 2001)

1 – 3 years – 2.5 mg/day (2,500 ppb) (NA, 2001)

4 – 8 years – 4.0 mg/day (4,000 ppb) (NA, 2001)

9 – 13 years males – 7.0 mg/day (7,000 ppb) (NA, 2001)

14 – 18 years males – 8.5 mg/day (8,500 ppb) (NA, 2001)

≥ 19 years males – 9.4 mg/day (9,400 ppb) (NA, 2001)

9 – 13 years females – 7.0 mg/day (7,000 ppb) (NA, 2001)

14 – 18 years females – 7.3 mg/day (7,300 ppb) (NA, 2001)

≥ 19 years females – 6.8 mg/day (6,800 ppb) (NA, 2001)

Pregnancy 14 – 18 years – 10.5 mg/day (10,500 ppb) (NA, 2001)

Pregnancy 19 – 50 years – 9.5 mg/day (9,500 ppb) (NA, 2001)

Lactation 14 – 18 years – 10.9 mg/day (10,900 ppb) (NA, 2001)

Lactation 19 – 50 years – 10.4 mg/day (10,400 ppb) (NA, 2001)

Tolerable Upper Intake Levels

Infants 0 – 6 months – 4 mg/day (4,000 ppb) (NA, 2001)

Infants 6 – 12 months – 5 mg/day (5,000 ppb) (NA, 2001)

1 – 3 years – 7 mg/day (7,000 ppb) (NA, 2001)

4 – 8 years – 12 mg/day (12,000 ppb) (NA, 2001)

9 – 13 years – 23 mg/day (23,000 ppb) (NA, 2001)

14 – 18 years – 34 mg/day (34,000 ppb) (NA, 2001)

≥ 19 years – 40 mg/day (40,000 ppb) (NA, 2001)

Pregnancy 14 – 18 years – 34 mg/day (34,000 ppb) (NA, 2001)

Pregnancy 19 – 50 years – 40 mg/day (40,000 ppb) (NA, 2001)

Lactation 14 – 18 years – 34 mg/day (34,000 ppb) (NA, 2001)

Lactation 19 – 50 years – 40 mg/day (40,000 ppb) (NA, 2001)

Standards and Guidelines
Minimum Risk Levels (MRL)

No Oral Acute duration was derived (ATSDR, 2005)

Oral intermediate duration – 0.3 mg/kg/day (300 ppb) (ATSDR, 2005)

Oral Chronic duration – 0.3 mg/kg/day (300 ppb) (ATSDR, 2005

Chapter 6 – Materials and Methods

The crayfish samples were procured in the Atchafalaya Basin and anthropogenic pond venues of South Central and South West Louisiana. The exoskeletons were removed and tail tissues were put into acid rinsed containers then filled with pure water. Afterwards, the holding containers were immediately put into a freezer for storage at 28ºF.

Samples were then transferred to the University of Louisiana at Lafayette engineering department where testing procedures were implemented on the tail tissues. Samples were defrosted in a laboratory refrigerator at approximately 40ºF.

The tail tissues were digested by microwave action. The samples were put individually into acid rinsed glass tubules. Ten mL of nitric acid were added to the tubule, and the tubule was filled to a designated fill line with pure water. Ten tissue and tubule samples were digested by microwave action. The digestion time was 15 minutes for complete digestion. The samples were then cooled for 20 minutes, and were ready for testing.

Testing commenced on each tissue sample from each tubule by Inductively Coupled Plasma-Mass Spectrometer (ICP-MS). Afterwards, the 10 test conclusions were recorded on a graph, interpreted, converted to numerical values in parts per billion, and micrograms per kilogram. The test results are reported in micrograms (µg/kg) for the 50 samples tested for arsenic, cadmium, copper, and zinc.

Chapter 6.1 – Apparatus and Materials

The procedure for the preparation of solids and solid type materials for analysis by ICP-MS employing the use of microwave assisted digestion is described herein.

Top loading balance capable of accurate weights to 0.001 g

Environmental Express Hot Block

Polypropylene digestion vessels: Environmental Express catalog # SC475

Reflux caps: Environmental Express catalog # SC506

Filtermate: Environmental Express catalog # SC 0401

Auto-pipettes or disposable glass pipettes

Plastic weighing boats

Boiling stones PTFE, 450 g bottle: Fisher Scientific catalog # 09-191-20

CEM MARS6 Microwave Reaction System

MARSXpress Plus vessels

Pure water (Elga Water Analyte-free): Evoqua Water Technologies LLC (Lowell, MA)

7700 Agilent ICP-MS Analyzer

Argon gas (high purity)

Helium gas (high purity)

Disposable polypropylene culture tubes (16 x 125 mm)

Computer system with Mass Hunter Workstation Software for ICP-MS, Version B.01.03 for data acquisition and manipulation

Chapter 6.2 – Reagents and Standards

Concentrated nitric acid (trace metals grade): Fisher Scientific catalog #A509-212

Concentrated hydrochloric acid: Fox Scientific Instrapure catalog # H300-3

30% Hydrogen peroxide: Fisher Scientific catalog #H325-50

Metals Standard 7: Environmental Express catalog # ICQ500-7

ICP-MS: Calibration Standard Stock/ICP-MS Spike

Standard: Place the amounts of each parent (designated in the table below) into a 500 mL volumetric flask. Add 20 mL HNO_3. Bring to volume using Elga water.

Table 1 – Reagents and Standards

Parent	Amount of Parent	Concentration
Aluminum (1000 ppm)	1.25 mL	2.5 ppm
Antimony (1000 ppm)	1.25 mL	2.5 ppm
Arsenic (1000 ppm)	1.25 mL	2.5 ppm
Beryllium (1000 ppm)	1.25 mL	2.5 ppm
Boron (1000 ppm)	1.25 mL	2.5 ppm
Cadmium (1000 ppm)	1.25 mL	2.5 ppm
Chromium (1000 ppm)	1.25 mL	2.5 ppm
Cobalt (1000 ppm)	1.25 mL	2.5 ppm
Copper (1000 ppm)	1.25 mL	2.5 ppm
Iron (1000 ppm)	1.25 mL	2.5 ppm
Lead (1000 ppm)	1.25 mL	2.5 ppm
Lithium (1000 ppm)	1.25 mL	2.5 ppm
Manganese (1000 ppm)	1.25 mL	2.5 ppm
Molybdenum (1000 ppm)	1.25 mL	2.5 ppm
Nickel (1000 ppm)	1.25 mL	2.5 ppm
Selenium (1000 ppm)	1.25 mL	2.5 ppm
Strontium (1000 ppm)	1.25 mL	2.5 ppm
Thallium (1000 ppm)	1.25 mL	2.5 ppm
Tin (1000 ppm)	1.25 mL	2.5 ppm
Zinc (1000 ppm)	1.25 mL	2.5 ppm

Use the above solution to make the MS/MSD by spiking with 1.0 mL of each standard to a final volume of 50 mL.

Chapter 6.3 – Digestion Procedure

Remove the crayfish tail from the sample jar into a plastic weighing boat. For each digestion procedure, weigh out whole crayfish tail or minimum of 1-2 grams of sample (weigh to the nearest 0.001 g) into a labeled polypropylene digestion vessel. The method used for fish parts, crayfish parts, or other biological samples is found under "Classic Methods" and labeled "Fish Digestion 3."

Power level of MARS6: set at 1500 watts

Ramp time: 15 minutes

Hold Time: 15 minutes

Temperature is set at 200ºC (not to exceed 260ºC)

Once digestion is complete and the samples have cooled, the sample is brought to a final volume of 50 mL with Elga water and then analyzed.

If the data must be reported based upon dry weight, determine the moisture content by the following: weigh out about 0.5 g of tissue into an aluminum pan (tare the weight of the pan before adding tissue). Record the total weight to 0.0001 g. Dry the sample in an oven at 103 – 105°C until weight loss is stable. Adjust the weight of sample extracted, for the moisture content by using the following formula:

$$\text{Dry Wt.} = \frac{(Wt_d - Wt_t)}{(Wt_w - Wt_t)} \times Wt_s$$

where Dry Wt. is weight as dry tissue, Wt_d is weight of dried pan and tissue, Wt_t is tare weight of aluminum pan, Wt_s is weight of wet tissue to be extracted, and Wt_w is the total weight of pan and wet tissue. All weights are in grams (g).

Chapter 6.4 – Quality Assurance/Quality Control (QAQC) for Digestion Procedure

A LCS, a method blank, a matrix spike, and a matrix spike duplicate are prepared for each batch of 20 samples.

Tissue Quality Assurance/Quality Control (QAQC) Samples:

LCS: Weigh out 1.0 g of Teflon boiling chips. Water and acids are added to the vessel, and spikes are chosen based on analyses required. Note: Add spiking solutions *before* acids (HNO_3 and HCl).

a. For ICP-MS: 1.0 mL of each solution in Chapter 6.2
b. Proceed with digestion as explained in Chapter 6.3

Matrix spike and matrix spike duplicates: are made by adding the desired spiking solution to the matrix sample as described above, and treating the samples as described in Chapter 6.2. Note: Add spiking solutions *before* acids are added.

Method blank: For soil samples should consist of 1.0 g of Teflon boiling chips and the acids HNO_3 and HCl, as well as the peroxide used in the digestion procedure.

The acceptability criteria for soils is method specified 75 – 125% recovery for LCS, MS/MSD.

In the event of MS/MSD failure, a post digestion spike (PDS) of the parent sample must be prepared and analyzed. As MS/MSD failure is common for metals analyses, it is recommended the analyst prepare a PDS along with the regular MS/MSD as part of the batch QAQC.

Preparation of the PDS: Remove an aliquot of the un-spiked parent sample digestate. Spike the digestate with the same amount and concentration of the MS/MSD spiking solution. If the

recovery of the PDS is within 85-115%, matrix effect should be suspected, and it becomes clear that the quantitation of the extract at the instrument was accurate.

If both the MS/MSD and PDS fail, matrix effects are confirmed.

If the analyte concentration is ≥ 10 times the reporting limit, a dilution test must be conducted. To conduct the dilution test, prepare a 1 to 5 dilution of the parent sample digestate. If the analysis of the 1 to 5 dilution does not agree within ±10% of the original determination, a chemical or physical interference should be suspected.

Chapter 6.5 – ICP-MS Analysis Procedure

ICP-MS Working Calibration Standards: Place the amount of the parent standard solution from Chapter 6.2 (designated in the table below) into a 50mL digestion tube. Add 2 mL of HNO_3. Bring to volume using Elga water. Repeat procedure for each standard. There 6 standards.

Table 2 – ICP-MS Calibration Standards (Working)

6 Standards	Amount of Parent	Concentration at Instrument in ppb
ICP-MS Calibration Standard Stock plus ICP-MS Silver Calibration Standard Stock	1.250 mL each	625.00
ICP-MS Calibration Standard Stock plus ICP-MS Silver Calibration Standard Stock	5.000 mL each	250.00
ICP-MS Calibration Standard Stock plus ICP-MS Silver Calibration Standard Stock	2.000 mL each	100.00
250 ppb from above	4.000 mL	20.00
250 ppb from above	0.400 mL	2.00
20 ppb from above	0.625 mL	0.25

Internal Standard Stock: Place 0.5 mL of each of the analytes below into Elga water. Add 20 mL of HNO_3 and bring to a final volume of 500 mL.

Table 3 – Analytes

Analyte	Catalog Number	Vendor	Original Concentration Purchased Standard	Final Concentration
Bismuth	HP10006-1	Environmental Express	1000 mg/L	1.0 mg/L
Holmium	HP10023-1	Environmental Express	1000 mg/L	1.0 mg/L
Indium	HP10024-1	Environmental Express	1000 mg/L	1.0 mg/L
Rhodium	HP10044-2	Environmental Express	1000 mg/L	1.0 mg/L
Scandium	HP10048-1	Environmental Express	1000 mg/L	1.0 mg/L
Terbium	HP10057-1	Environmental Express	1000 mg/L	1.0 mg/L
Yttrium	HP10067-1	Environmental Express	1000 mg/L	1.0 mg/L

Internal Standard Working Solution: Place 25 mL of Internal Standard Stock with 10 mL HNO_3 and bring to 250 mL with Elga water.

Table 4 – ICP-MS Agilent Stock Standard Tuning Solutions

Analyte	Concentration
Cesium	1 mg/L
Cobalt	1 mg/L
Lithium	1 mg/L
Thallium	1 mg/L
Yttrium	1 mg/L

Table 5 – Agilent 7500 Series PA Tuning Stock Standard Solutions 1 and 2

PA Solution 1				PA Solution 2	
Analyte	**Concentra-tion**	**Analyte**	**Concentra-tion**	**Analyte**	**Concentra-tion**
Arsenic	20.0 mg/L	**Bismuth**	5 mg/L	**Germanium**	10 mg/L
Beryllium	20.0 mg/L	**Cobalt**	5 mg/L	**Molybdenum**	10 mg/L
Cadmium	20.0 mg/L	**Chromium**	5 mg/L	**Ruthenium**	10 mg/L
Zinc	20.0 mg/L	**Copper**	5 mg/L	**Antimony**	10 mg/L
Magnesium	10.0 mg/L	**Indium**	5 mg/L	**Tin**	10 mg/L
Nickel	10.0 mg/L	**Lithium**	5 mg/L	**Iridium**	5 mg/L
Lead	10.0 mg/L	**Lutetium**	5 mg/L	**Titanium**	5 mg/L
Aluminum	5.0 mg/L	**Manganese**	5 mg/L	**Palladium**	10 mg/L
Barium	5.0 mg/L	**Sodium**	5 mg/L		
Strontium	5.0 mg/L	**Scandium**	5 mg/L		
Thallium	5.0 mg/L	**Thorium**	5 mg/L		
Uranium	5.0 mg/L	**Titanium**	5 mg/L		
Yttrium	2.5 mg/L	**Vanadium**	5 mg/L		
Ytterbium	2.5 mg/L				

Table 6 – ICP-MS 200.8 Stock Parent Tuning Solution

Analyte	Concentration
Beryllium	1,000 mg/L
Cobalt	1,000 mg/L
Indium	1,000 mg/L
Lead	1,000 mg/L
Magnesium	1,000 mg/L

Agilent Working Tune Standard: The standard is prepared fresh as needed from the purchased standard described in Table 4. 50 µL of the stock standard and 20 mL HNO_3 is brought to a final volume of 500 mL with Elga water in a class A volumetric.

Agilent 7500 Series PA Working Tune Standard: The standard is prepared fresh as needed from the purchased standard described in Table 5. Standard PA 1 PA 2 (6.66 mL each) and 20 mL HNO_3 are brought to a final volume of 500 mL in a class A volumetric with Elga water.

ICP-MS 200.8 Tune-Stability Working Solution: The standard is prepared from a stock parent solution described in Table 6 and from the ICP-MS Agilent stock standard tuning solution. 0.5 mL of each standard with 20 mL HNO_3 is brought to a final volume of 500 mL in a class A volumetric with Elga water.

Chapter 6.6 – Quality Assurance/Quality Control (QAQC) for ICP-MS Procedure

Each analytical batch requires the preparation and analysis of a method blank, LCS, MS, and MSD. Method blank for water samples consists of Elga water containing 4% concentrated nitric acid. Method blank for soil samples consists of Elga water containing 10% nitric acid. Calibration blank is prepared using 48 mL Elga water and 2 mL of concentrated nitric acid to a final volume of 50 mL.

In the case of MS/MSD failure, a post-digestion spike is prepared and analyzed. A MS/MSD failure is common for metals analyses, and it is recommended the analyst prepare a post-digestion spike along with the regular MS and MSD as part of the batch QAQC.

Preparation of the Post Digestion Spike (PDS): Remove an aliquot of the un-spiked parent sample digestate. Spike the digestate with the same amount and concentration of the MS/MSD spiking solution. If the recovery of the PDS is greater than +15% of the expected value, matrix effect should be suspected. If the recovery of the PDS is within +15%, the quantitation of the extract at the instrument was accurate.

If both the MS/MSD and PDS fail, matrix effects are confirmed. If the analyte concentration is sufficiently high (minimally a factor of 10 above the lower limit of quantitation after dilution), a dilution test must be conducted.

To conduct the dilution test, prepare a 1 to 5 dilution of the parent sample digestate. If the analysis of the 1 to 5 dilution does not agree within +10% of the original determination, a chemical or physical interference should be suspected.

Table 7 – Summary, Required Quality Control, Frequency, and Acceptable Limits

Procedure	Frequency	Acceptable Limits
ICV	Daily after the initial calibration **Low Level ICV**: same source as Cal. Stds.; concentration at Quantitation Limit of water **Mid Level ICV**: 2nd source	**Low Level ICV:** 70 – 130% **Mid Level ICV:** % Rec. ±10% of T.V. as per Method 200.8
CCV	**Mid Level CCV:** After every 10 samples and at the end of the run. Second source **Low Level CCV:** Concentration at the Quantitation Limit of water. Analyzed at the beginning and end of batch at a minimum (same source as calibration standards)	**Mid Level CCV:** % Rec. ±10% of t.V. as per Methods 200.8 and 6020A **Low Level CCV:** 70 – 130%
Calibration Blank	Run as part of the calibration curve and also every 10 samples	Analytes must be less than Reporting Limit
Method Blank	One per sample batch; used to identify possible contamination	Analytes must be less than Reporting Limit
LCS	At least one LCS per batch of samples; two should be used to track precision if a MS/MSD pair is not used. Second Source	% Rec. 85 – 115 (water) as per Method 200.8
MS	One per batch of 20 samples	%Rec. 75 – 125% as per 6020A
MSD	One per batch of 20 samples except TCLP	RPD 20% as per 6020A
Post Digestion Spike (PDS)	Analyzed when BOTH MS and MSD fail	%Rec. 85 – 115; If failure occurs, analyze a 1/5 dilution of parent digestate
Dilution Test	Analyze a 1/5 dilution of parent digestate in the event of PDS failure IF the parent sample contains analyte concentration at least 10X the limit of quantitation	If 1/5 digestate results are within ±10% of UNDILUTED digestate, matrix effect ruled out; if > ±10% difference, matrix effects confirmed
Interference Check Sample	Analyzed at the beginning of each sample run	75 – 125% of T.V.
Lower Limit of Quantitation Check Sample (LLQC)	Analyzed annually to verify the reporting limit. Spike a QAQC at the reporting limit of water. Prep and analyze as a regular sample using the same source as calibration standards.	50 – 150%

Note to Table 7: Acceptance criteria for water are method defined: LCS 85 – 115% recovery; MS/MSD 75 – 125% Recovery; 20% RPD. The acceptability criteria for soil are method specified: 80 – 120% recovery for LCS; 75 – 125% recovery MS/MSD. The criteria used for wipes and filters are the same for soils.

Chapter 6.7 – Materials and Methods References

SW-846: Method 6020A, Revision 1, February 2007

EPA Methods for Chemical Analysis of Water and Wastewater Method 200.8, Revision 5.4 (1994), EMMC Version

Chapter 7 – Test Results and Statistics

Chapter 7.1 – Arsenic Test Results and Statistics – Tail with Intestine

Table 8 – Tail with Intestine

Sample Name	Result (µg/kg)	Detection Limit	Reference Limit	Dilution
ATCH-B-1	28.30	1.730	2.89	1
ATCH-B-2	85.30	1.300	2.17	1
ATCH-B-3	28.30	1.110	1.84	1
ATCH-B-4	48.90	1.260	2.11	1
ATCH-B-5	29.30	1.120	1.86	1
ATCH-B-6	52.00	1.500	2.49	1
ATCH-B-7	27.80	0.976	1.63	1
ATCH-B-8	43.70	1.590	2.66	1
ATCH-B-9	50.00	1.460	2.43	1
ATCH-B-10	17.30	1.110	1.85	1
ATCH Extra	29.50	1.900	3.17	1
ATCH Extra & 11 TS	23.90	1.610	2.69	1
CHI-1	216.00	14.600	24.30	10
CHI-2	84.10	14.000	23.30	10
CHI-3	74.50	15.700	26.20	10
CHI-4	151.00	16.300	27.20	10
CHI-5	93.00	12.100	20.10	10
CHI-6	204.00	13.400	22.30	10
CHI-7	241.00	18.300	30.50	10
CHI-8	188.00	14.800	24.70	10
CHI-9	90.90	15.800	26.40	10
CHI-10	96.60	11.800	19.70	10
DOM-1-1	191.00	1.230	2.05	1
DOM-1-2	<2.13	1.280	2.13	1
DOM-1-3	80.70	8.990	15.00	10
DOM-1-4	72.30	10.900	18.20	10
DOM-1-5	104.00	9.960	16.60	10
DOM-1-6	93.70	7.360	12.30	10
DOM-1-7	66.30	9.760	16.30	10
DOM-1-8	89.70	9.920	16.50	10
DOM-1-9	75.10	12.600	21.00	10
DOM-1-10	74.40	11.000	18.40	10
DOM-2-1	104.00	15.700	26.20	10
DOM-2-2	73.20	13.500	22.50	10
DOM-2-3	90.80	17.200	28.70	10
DOM-2-4	66.10	11.100	18.50	10
DOM-2-5	74.30	13.500	22.50	10
DOM-2-6	53.60	13.700	22.80	10

(Table 8 cont.)

Sample Name	Result (µg/kg)	Detection Limit	Reference Limit	Dilution
DOM-2-7	92.40	14.300	23.80	10
DOM-2-8	84.70	20.200	33.60	10
DOM-2-9	72.30	18.300	30.50	10
DOM-2-10	98.10	15.200	25.30	10
FRE-1	72.10	1.490	2.49	1
FRE-2	102.00	1.050	1.75	1
FRE-3	83.30	1.510	2.52	1
FRE-4	69.40	1.210	2.02	1
FRE-5	79.60	0.873	1.45	1
FRE-6	58.50	0.949	1.58	1
FRE-7	107.00	0.986	1.64	1
FRE-8	84.60	1.230	2.05	1
FRE-9	90.50	1.100	1.84	1
FRE-10	94.30	1.250	2.09	1
FRE-Extra	83.70	1.370	2.28	1

The Means Procedure

This provides a data summary computing descriptive statistics for the variables across groups of observations (ATCH-B, CHI, DOM-1, DOM-2, FRE, and Intestine). It provides the number of observations, the mean, standard deviation, minimum value, and maximum values for all the observations and between groups.

Welch's ANOVA Procedure

The analysis of variance procedure (ANOVA) performs analysis of variance, which measures the variation of the response variable across the groups. Caution: ANOVA procedure in SAS is used for balanced data. Since this data is unequal across test groups, the validity of the results are questionable.

The GLM Procedure

The generalized linear model procedure (GLM) in SAS uses least squares to fit linear models. Since the dependent variables used here are classification groups, the analysis becomes

analysis of variance. This type of analysis of variance *can* handle unbalanced data. This output gives the same results as the ANOVA procedure, but also gives type I and type II sums of squares. These sums of squares deal with the model being assumed and since there is only one factor at four levels, both types of sums of squares is equal to the model sum of squares. The GLM procedure also tests for the model assumption of homogeneity. Homogeneity is the equality of variances. The GLM Procedure uses Levene's Test to validate homogeneity.

The Univariate Procedure

The univariate procedure provides a Q-Q Plot of the residuals verses the quantiles of the data observations. This is necessary to validate the ANOVA model. Normality of the observations is an important model assumption and validated by a Q-Q Plot.

***T* Tests (LSD) for Result**

Fisher's least significant difference test (LSD) compares the mean of one group to the means of another at a certain significance level using the *t* distribution to determine if the responses from two different groups differ significantly.

Tukey's Studentized Range (HSD) Test for Results

Tukey's honest significant difference (HSD) compares the means of one group to the means of another using a studentized range distribution. It is mainly used when there are unequal sample sizes.

Conclusions from Data

With 53 observations, the mean response variable across all groups is 84.66 with a standard deviation of 48.87. The minimum value is 2.13 and the maximum is 241. The ATCH-B group has 12 observations with a mean of 38.69, a standard deviation of 18.56, a minimum value of 17.3, and a maximum value of 85.30. The CHI group has 10 observations with a mean of

149.91, a standard deviation of 63.48, a minimum value of 74.5 and a maximum value of 241.00. The DOM-1 group has 10 observations with a mean of 84.93, a standard deviation of 46.31, a minimum value of 2.13, and a maximum value of 191.00. The DOM-2 group has 10 observations with a mean of 80.95, a standard deviation of 15.69, a minimum value of 53.60, and a maximum value of 104.00. The FRE group has 11 observations with a mean of 84.09, a standard deviation of 14.24, a minimum value of 58.50, and a maximum value of 107.00. The intestine group has 5 observations with a mean of 340.26, a standard deviation of 250.26, a minimum value of 54.30, and a maximum value of 668.00.

The means of five groups (ATCH-B, CHI, DOM-1, DOM-2, and FRE) were tested using ANOVA to see if the groups were significantly different. The model used is assumed to be $y_{ij} = \mu + \tau_i + \epsilon_{ij}$, where y_{ij} is an individual observation, μ is the overall mean, τ_i is treatment effect, and ϵ_{ij} is the random error. ANOVA tests the equality of treatment means. Since the *p* value is less than 0.0001, this suggests that one or more of the groups are significantly different. Since ANOVA is valid for equal sample sizes, GLM performed to account for unequal sample sizes. The *p* value is still less than 0.0001, which further validates that the groups significantly differ. This is also easily seen on the box plot. The R^2 value in the GLM is 0.488, meaning 48.8% of the response variables can be explained or predicted by the groups.

Comparisons of one group to another were made by *t* tests and Tukey's studentized range tests. It was found that CHI and ATCH-B groups were significantly different than the other groups. The Tukey's studentized range concludes that ATCH-B is only significantly different from CHI, DOM-1, and FRE, not DOM-2. This difference in conclusions is due to the unequal sample sizes.

Model assumptions must be valid for these results to hold true. The two assumptions are homogeneity and normality of the data. Homogeneity means equal variances across all groups and is determined by Levene's test which compares the variances of each group. Since the *p* value of this test is below the significance value, the null hypothesis of the variances being equal can be rejected. Hence, the variances are not the same and homogeneity cannot be assumed. This can be seen in the box plots very easily. It appears that the variance of CHI differs significantly compared to the other groups. Caution is recommended when forming conclusions on CHI group. A quantile-quantile plot is used to check normality. The Q-Q plot of the residuals appears linear, so normality of the data set holds.

In conclusion, ATCH-B was significantly lower than the rest of the groups, with a mean of 38.69 while CHI was significantly higher with a mean of 143.91. The variance of CHI was also much higher than any of the tests groups which could have contributed to the failing of Levene's test. Responses from groups DOM-1, DOM-2, and FRE did not significantly differ from each other.

Chapter 7.2 – Arsenic Test Results and Statistics – Intestine

Table 9 – Intestine

Sample Name	Result (µg/kg)	Detection Limit	Reference Limit	Dilution
ATCH-B	54.3	24.90	41.50	1
CHI	668.0	14.90	24.80	1
DOM-1	135.0	49.30	82.20	1
DOM-2	476.0	3.74	6.24	1
FRE	368.0	20.20	33.60	1

The *T* Test Procedure

This procedure performs *t* tests and calculates confidence limits (CI) for one sample, paired observations, or two independent samples.

The Means Procedure

This provides a data summary computing descriptive statistics for the variables across groups of observations (ATCH-B, CHI, DOM-1, DOM-2, FRE, and Intestine). It provides the number of observations, the mean, standard deviation, minimum value, and maximum values for all the observations and between groups.

Arsenic Statistical Analysis Results

Intestine type N group was composed of 53 samples with a mean of 84.67, a standard deviation of 48.87, a standard error of 6.71, a minimum value of 2.13, and a maximum value of 241.00. Ninety-five percent of the samples were located between 71.19 and 98.14. The intestine type Y group was composed of five samples with a mean of 340.3, a standard deviation of 250.3, a standard error of 111.9, a minimum value of 54.30, and a maximum value of 668.00. Ninety-five percent of intestine type Y's samples were located between 29.53 and 651.0. The groups' difference had an average of -255.6, a standard deviation of 81.80, and a standard error of 38.2691.

For testing, if the means are significantly different, two assumptions must be satisfied: the variances of the two groups must be equal and the data must follow a normal distribution. A pooled *t* test was performed to test the difference of the means. A pooled *t* test assumes the variances are equal, all the samples are independent, and each sample is sampled from a population that is approximately normally distributed. The *p* value for this test is less than 0.0001, which is less than the significance level of 0.05. This suggests the means of the two groups are significantly different, having a higher arsenic concentration in the Y group than the N group. The Satterthwaite procedure is a way of testing means with unequal variances. The *p* value for this test was computed as 0.0843 which is greater than the significance level of 0.05. The Satterthwaite holds stronger inferences if the variances are unequal.

An f-test is used to test for equality of variances, but it is extremely sensitive to normality assumptions. The *p* value for this test is less than 0.0001, so it can be concluded that the variances are not equal. This concludes that the Satterthwaite holds stronger conclusions that the two tail groups do not significantly differ.

The distributions for the two groups were checked by plotting and fitting normal distributions and a composing a Q-Q plot. The results of the distribution for the N group do not appear normal. Deviations from the Q-Q plot exist and the distribution appears to be skewed. This could be fixed by transforming the data or other tests could be performed for more robust inferences for a skewed distribution. The distribution for the Y tail group also deviates from normality, which could be fixed by having a higher sample size. Since both groups depart from normality, the f-test does not hold as strong, and the Satterthwaite may not offer a certain conclusion. From initial observations of the difference in means and the initial results of the pooled *t* test, further investigations on this subject should be made.

Chapter 7.3 – Arsenic Statistical Report

Arsenic concentration was analyzed in 53 tail samples. The sample mean, sample standard deviation, the minimum, and the maximum values are reported in the table below.

Table 10 – Analysis Variable Results – Total Samples

N	Mean	Std Dev	Minimum	Maximum
53	84.67	48.87	2.13	241.00

The table below summarizes the key statistics for arsenic in the tails with intestine by the site.

Table 11 – Analysis Variable Results – Tail with Intestine

Sample Name	N	Mean	Std Dev	Minimum	Maximum
ATCH-B	12	38.69	18.56	17.30	85.30
CHI	10	143.91	63.48	74.50	241.00
DOM-1	10	84.93	46.31	2.13	191.00
DOM-2	10	80.95	15.69	53.60	104.00
FRE	11	84.09	14.24	58.50	107.00

The table below summarizes the key statistics for the intestine.

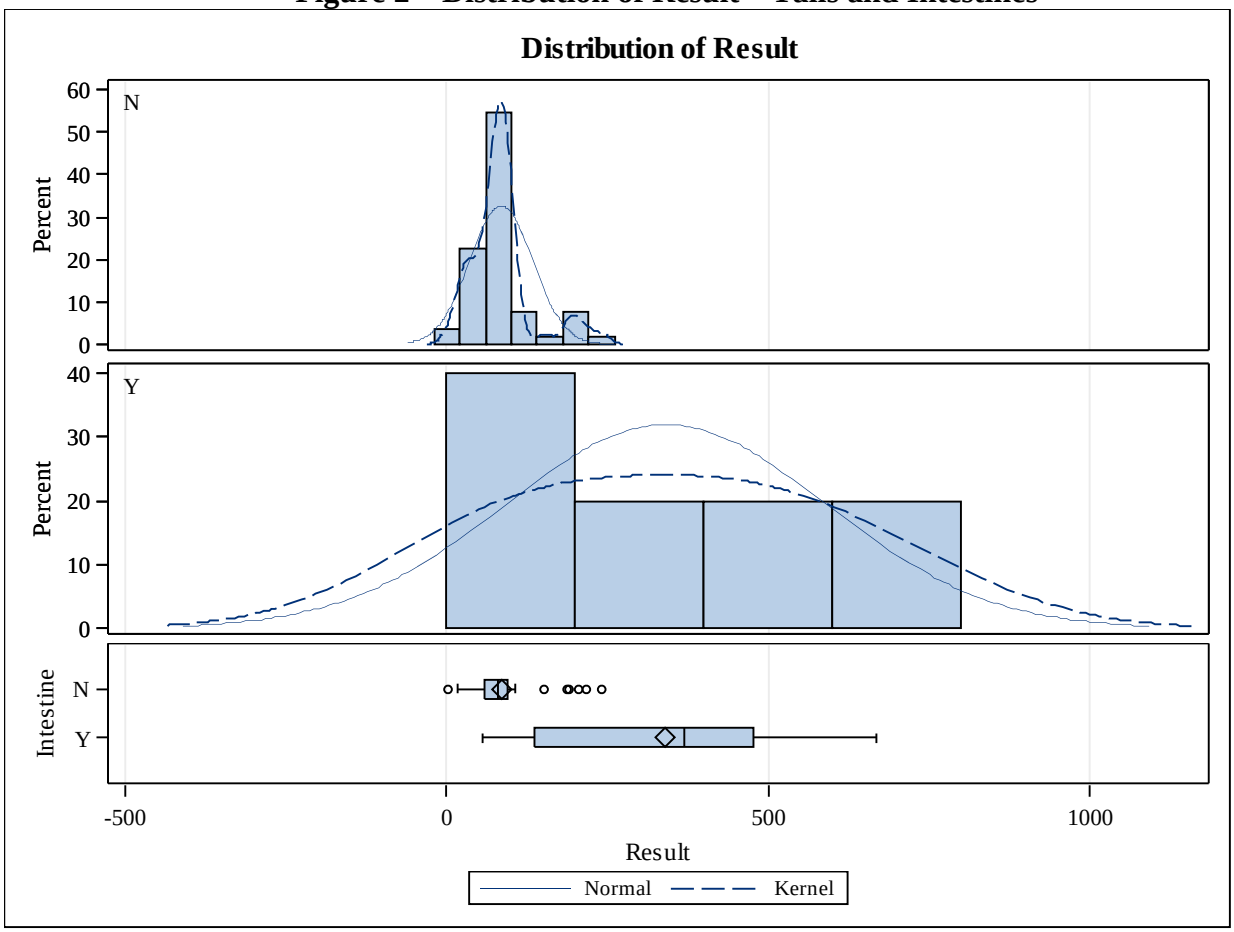
Table 12 – Analysis Variable Results – Intestine

Y	Mean	Std Dev	Minimum	Maximum
5	340.26	250.26	54.30	668.00

Statistical Comparison of tails and Intestines: Note that there are 53 readings for the tails, whereas there are only five for the intestine. In the plots, the variable *gut type* is coded as Y for the arsenic in intestines and as N for the arsenic in tails. The figure first summarizes the statistical distribution for these two populations. Note that graphically the fitted normal distribution seems normal for the tails, but not so for the intestines. With only five observations from the intestines, it is quite difficult to form a convincing conclusion. The figure below also

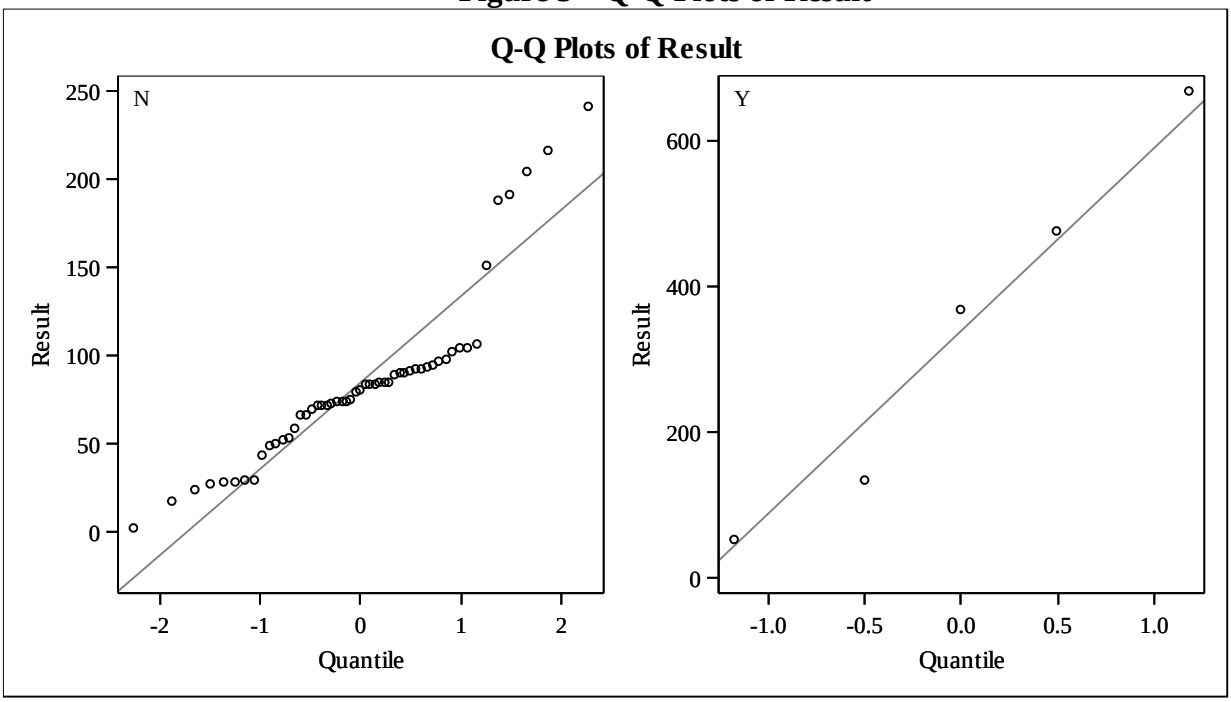
depicts boxplots for the tails and intestines. The boxplot for tails depicts the potential outlier arsenic observations. This is studied further via GLM.

Figure 2 – Distribution of Result – Tails and Intestines



A QQ plot shows mild departure from normality. Note that the sample size of tails is moderately large and the central limit theorem will ensure the normality requirement for the statistical tests provided below.

Figure 3 – Q-Q Plots of Result



The comparisons of the means for the arsenic in the crayfish tails versus intestines is carried out next using the *t* test. The mean arsenic in the tails is about 255.6 µg/kg smaller than the mean arsenic in the intestine.

Table 13 – *T* Test

Type	N	Mean	Std Dev	Std Err	Minimum	Maximum
Tails with Intestine	53	84.67	48.87	6.71	2.13	241.00
Intestine	5	340.30	250.30	111.90	54.30	668.00
Difference		-255.60	81.80	38.27		

The table below provides 95% confidence limits (CL) for the mean arsenic contents in the tails and intestines. The table also provides a 95% confidence limits for the standard deviations. The mean differences are pooled, which assumes equal variances for tails and intestines, and the Satterthwaite are provided in the table below. The Satterthwaite test uses the Satterthwaite approximation for degrees of freedom. The confidence limits for the standard deviations are of the equal-tailed variety. Note that the sample standard deviation for the arsenic in the tails is 48.87 µg/kg, whereas for intestines it is 250.3 µg/kg. This difference in standard deviations (or variances) is tested via a folded F-Test and the test concludes that the variances are unequal with *p* value of <0.0001.

Table 14 – 95% Confidence Limits

Type	Method	Mean	95% CL Mean		Std Dev	95% CL Std Dev	
Tails		84.67	71.19	98.14	48.87	41.02	60.47
Intestine		340.30	29.53	651.00	250.30	149.90	719.10
Difference	Pooled	-255.60	-332.30	-178.90	81.80	69.06	100.30
Difference	Satterthwaite	-255.60	-566.00	54.82			

The tables below provide the *p* values for the difference in the mean arsenic contents between tails and intestines under both equal and unequal variances. Assuming the variances are equal, the *p* value of the difference is highly significant (*p* value <0.0001); assuming that the variances are unequal, the *p* value is 0.0843.

Table 15 – *P* values

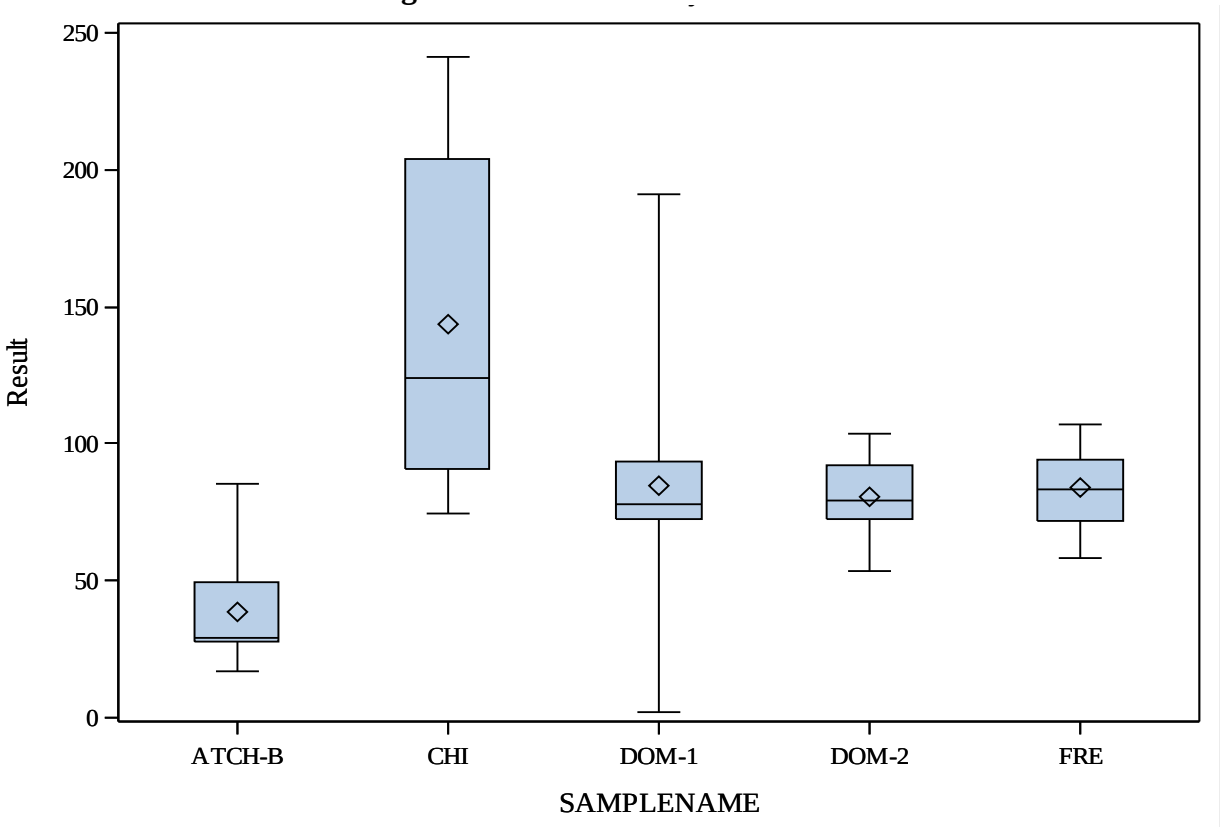
Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	56.00	-6.68	<.0001
Satterthwaite	Unequal	4.03	-2.28	0.0843

Table 16 – P-value – Equality of Variances

Method	Num DF	Den DF	F Value	Pr > F
Folded F	4	52	26.22	<.0001

Statistical Comparison of Tails: We have collected 53 observations on the arsenic contents in the tails from five sites. The figure below shows the boxplots of arsenic content by site.

Figure 4 – Distribution of Results – Tails



A quick visual inspection shows that ATCH-B site has the lowest arsenic in the tails, and CHI the highest. The other three sites, DOM-1, DOM-2, and FRE are quite similar in arsenic contents in the tails. Next, via a GLM, an unbalanced ANOVA model is fitted.

First, we test the assumption of the equality of the variances by Levene's Test. Note that the Levene's Test rejects the assumption of homogeneity of variance with a *p* value of <0.0001.

Table 17 – Levene's Test for Homogeneity of Result Variance
ANOVA of Absolute Deviations from Group Means

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Sample Name	4	14730.7	3682.7	9.29	<.0001
Error	48	19030.6	396.5		

Since the assumption of homogeneity of variance is suspect, we report the results of the ANOVA test which is robust to violation of the assumption of equal variances for one-way models.

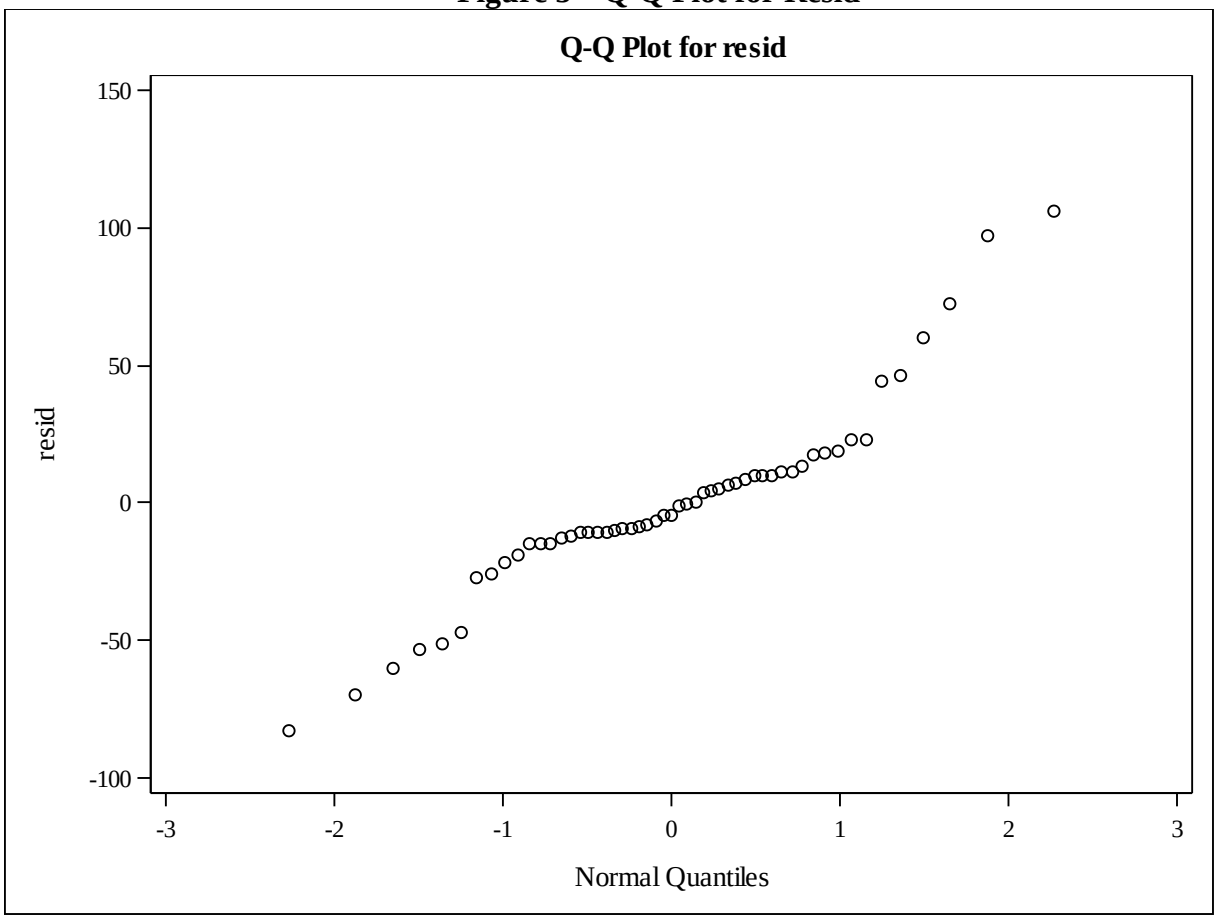
Table 18 – Welch's ANOVA for Result

Source	DF	F Value	Pr > F
Sample Name	4.0000	14.56	<.0001
Error	22.6836		

The ANOVA test concludes that there is a statistically significant difference in the mean arsenic in tails from the five sites with a *p* value of <0.0001.

The QQ plot of the residuals plotted below does not show large departures from the normality.

Figure 5 – Q-Q Plot for Resid



Tukey's Studentized Range (HSD) Test (Post Hoc): The post hoc tests are designed for further investigation into the situations when the F-Test is significant and the researcher is interested in which means are significantly different from each other. Tukey's studentized range test corrects for multiple tests so that a correct Type I experiment error rate is maintained, even though multiple tests or comparisons are computed.

The tables below summarize the Tukey's test and statistically significant differences at the 5% level are indicated below.

Table 19 – Turkey's Studentized Range (HSD) Test

Alpha	0.05000
Error Degrees of Freedom	48.00000
Error Mean Square	1324.95200
Critical Value of Studentized Range	4.00812

Table 20 – Turkey's Studentized Range (HSD) Test

Comparisons significant at the 0.05 level are indicated by ***				
Sample Name Comparison	**Difference Between Means**	**Simultaneous 95% Confidence Limits**		
CHI – DOM-1	58.98	12.84	105.11	***
CHI – FRE	59.82	14.74	104.89	***
CHI – DOM-2	62.96	16.82	109.10	***
CHI – ATCH-B	105.22	61.05	149.39	***
DOM-1 – CHI	-58.98	-105.11	-12.84	***
DOM-1 – FRE	0.84	-44.23	45.92	
DOM-1 – DOM-2	3.98	-42.15	50.12	
DOM-1 – ATCH-B	46.24	2.07	90.41	***
FRE – CHI	-59.82	-104.89	-14.74	***
FRE – DOM-1	-0.84	-45.92	44.23	
FRE – DOM-2	3.14	-41.93	48.22	
FRE – ATCH-B	45.40	2.34	88.46	***
DOM-2 – CHI	-62.96	-109.10	-16.82	***
DOM-2 – DOM-1	-3.98	-50.12	42.15	
DOM-2 – FRE	-3.14	-48.22	41.93	
DOM-2 – ATCH-B	42.26	-1.91	86.43	
ATCH-B – CHI	-105.22	-149.39	-61.05	***
ATCH-B – DOM-1	-46.24	-90.41	-2.07	***
ATCH-B – FRE	-45.40	-88.46	-2.34	***
ATCH-B – DOM-2	-42.26	-86.43	1.91	

Summary and Limitations: Due to small sample size for intestines and the CHI site showing some unusually high levels of arsenic in the tails, the difference between tails and intestines is mildly statistically significant.

There is a strong statistical difference between the five sites. The arsenic content in DOM-1, DOM-2, and FRE is not statistically significant. CHI is significantly larger than these three sites. ATCH-B has the lowest arsenic content.

Chapter 7.4 – Cadmium Test Results and Statistics – Tail with Intestine

Table 21 – Tail with Intestine

Sample Name	Result (µg/kg)	Detection Limit	Reference Limit	Dilution
ATCH-B-1	<2.89	1.730	2.89	1
ATCH-B-2	<2.17	1.300	2.17	1
ATCH-B-3	<1.84	1.110	1.84	1
ATCH-B-4	<2.11	1.260	2.11	1
ATCH-B-5	<1.86	1.120	1.86	1
ATCH-B-6	<2.49	1.500	2.49	1
ATCH-B-7	<1.63	0.976	1.63	1
ATCH-B-8	<2.66	1.590	2.66	1
ATCH-B-9	<2.43	1.460	2.43	1
ATCH-B-10	<1.85	1.110	1.85	1
ATCH Extra	<31.70	19.000	31.70	10
ATCH Extra & 11 TS	<2.69	1.610	2.69	1
CHI-1	<24.30	14.600	24.30	10
CHI-2	<23.30	14.000	23.30	10
CHI-3	<26.20	15.700	26.20	10
CHI-4	<27.20	16.300	27.20	10
CHI-5	<20.10	12.100	20.10	10
CHI-6	<22.30	13.400	22.30	10
CHI-7	<30.50	18.300	30.50	10
CHI-8	<24.70	14.800	24.70	10
CHI-9	<26.40	15.800	26.40	10
CHI-10	<19.70	11.800	19.70	10
DOM-1-1	<2.05	1.230	2.05	1
DOM-1-2	<2.13	1.280	2.13	1
DOM-1-3	<15.00	8.990	15.00	10
DOM-1-4	<18.20	10.900	18.20	10
DOM-1-5	<16.60	9.960	16.60	10
DOM-1-6	<12.30	7.360	12.30	10
DOM-1-7	<16.30	9.760	16.30	10
DOM-1-8	<16.50	9.920	16.50	10
DOM-1-9	<21.00	12.600	21.00	10
DOM-1-10	<18.40	11.000	18.40	10
DOM-2-1	<26.20	15.700	26.20	10
DOM-2-2	<22.50	13.500	22.50	10
DOM-2-3	<28.70	17.200	28.70	10
DOM-2-4	<18.50	11.100	18.50	10
DOM-2-5	<22.50	13.500	22.50	10
DOM-2-6	<22.80	13.700	22.80	10
DOM-2-7	<23.80	14.300	23.80	10
DOM-2-8	<33.60	20.200	33.60	10

(Table 21 cont.)

Sample Name	Result (µg/kg)	Detection Limit	Reference Limit	Dilution
DOM-2-9	<30.50	18.300	30.50	10
DOM-2-10	<25.30	15.200	25.30	10
FRE-1	<2.49	1.490	2.49	1
FRE-2	<1.75	1.050	1.75	1
FRE-3	<2.52	1.510	2.52	1
FRE-4	<2.02	1.210	2.02	1
FRE-5	<1.45	0.873	1.45	1
FRE-6	<1.58	0.949	1.58	1
FRE-7	<1.64	0.986	1.64	1
FRE-8	<2.05	1.230	2.05	1
FRE-9	<1.84	1.100	1.84	1
FRE-10	<2.09	1.250	2.09	1
FRE-Extra	<2.28	1.370	2.28	1

The Means Procedure

With 53 observations, there is a mean of 4.05, a standard deviation of 3.35, a minimum value of 0.44, and a maximum value of 10.10. The group ATCH-B has 12 observations with a mean of 1.41, a standard deviation of 2.55, a minimum value of 0.49, and a maximum value of 9.50. The group CHI has 10 observations with a mean of 7.34, a standard deviation of 0.99, a minimum value of 5.90, and a maximum value of 9.15. The group DOM-1 includes 10 observations, with a mean of 4.15, a standard deviation of 1.98, a minimum value of 0.62, and a maximum value of 6.30. The group DOM-2 has 10 observations with a mean of 7.64, a standard deviation of 1.34, a minimum value of 5.55, and a maximum value of 10.10. The group FRE has 11 observations with a mean of 0.59, a standard deviation of 0.11, a minimum value of 0.44, and a maximum value of 0.76. The group intestine has 5 observations with a mean of 16.20, a standard deviation of 8.83, a minimum value of 7.20, and a maximum value of 26.60.

Other Procedures

ANOVA is used to verify differences between groups. Five groups (ATCH-B, CHI, DOM-1, DOM-2, and FRE are used for this analysis. The *p* value is below 0.0001, which is below the significance level of 0.05. This concludes that at minimum, one group differs. The R^2 value in the GLM is 0.774, which demonstrates that 77.4% of the response variables can be explained or predicted by the groups. From the box plot distribution of results, it seems that ATCH-B and FRE differ from CHI, DOM-1, and DOM-2. It is possible DOM-1 significantly differs from all the other groups. Individual *t* tests should be performed to validate these assumptions. Also, equality of variance tests and normality tests should be completed to draw correct inferences.

Chapter 7.5 – Cadmium Test Results and Statistics – Intestine

Table 22 – Intestine

Sample Name	Result (µg/kg)	Detection Limit	Reference Limit	Dilution
ATCH-B	7.20	3.74	6.24	1
CHI	<82.20	49.30	82.20	1
DOM-1	26.60	14.90	24.80	1
DOM-2	<33.60	20.20	33.60	1
FRE	<41.50	24.90	41.50	1

Chapter 7.6 – Cadmium Statistical Report

Fifty-three samples of tails were analyzed for cadmium. The sample mean, sample standard deviation, the minimum, and the maximum values are reported in the table below.

Table 23 – Analysis Variable Results – Total Samples

N	Mean	Std Dev	Minimum	Maximum
53	4.05	3.35	0.44	10.10

The table below summarizes the key statistics for cadmium in the tails with intestine by the site.

Table 24 – Analysis Variable Results – Tail with Intestine

Sample Name	N	Mean	Std Dev	Minimum	Maximum
ATCH-B	12	1.41	2.559	0.49	9.50
CHI	10	7.34	0.990	5.90	9.15
DOM-1	10	4.15	1.980	0.62	6.30
DOM-2	10	7.64	1.340	5.56	10.10
FRE	11	0.59	0.110	0.44	0.76

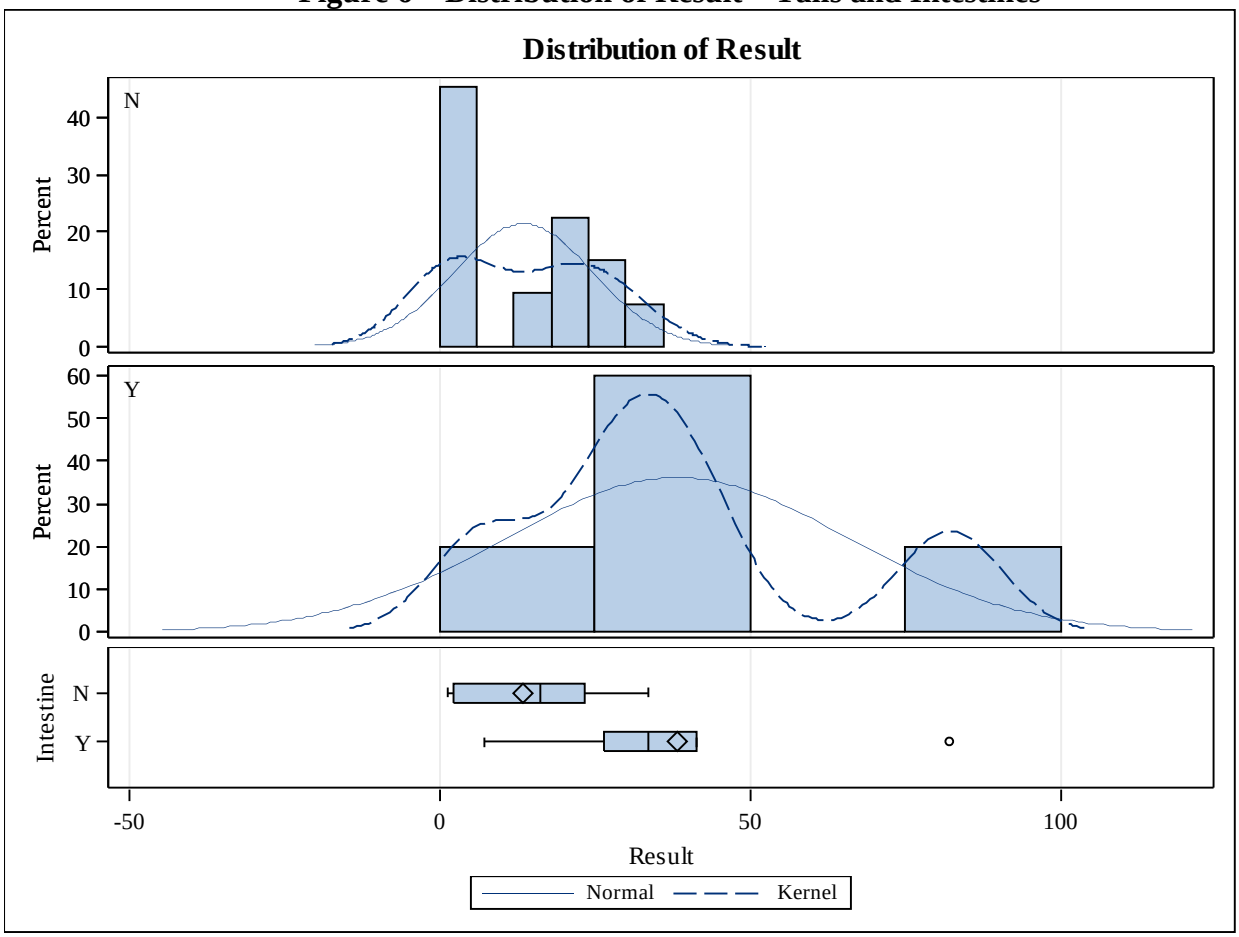
The table below summarizes the key statistics for the intestine.

Table 25 – Analysis Variable Results – Intestine

N	Mean	Std Dev	Minimum	Maximum
5	16.20	8.83	7.20	26.60

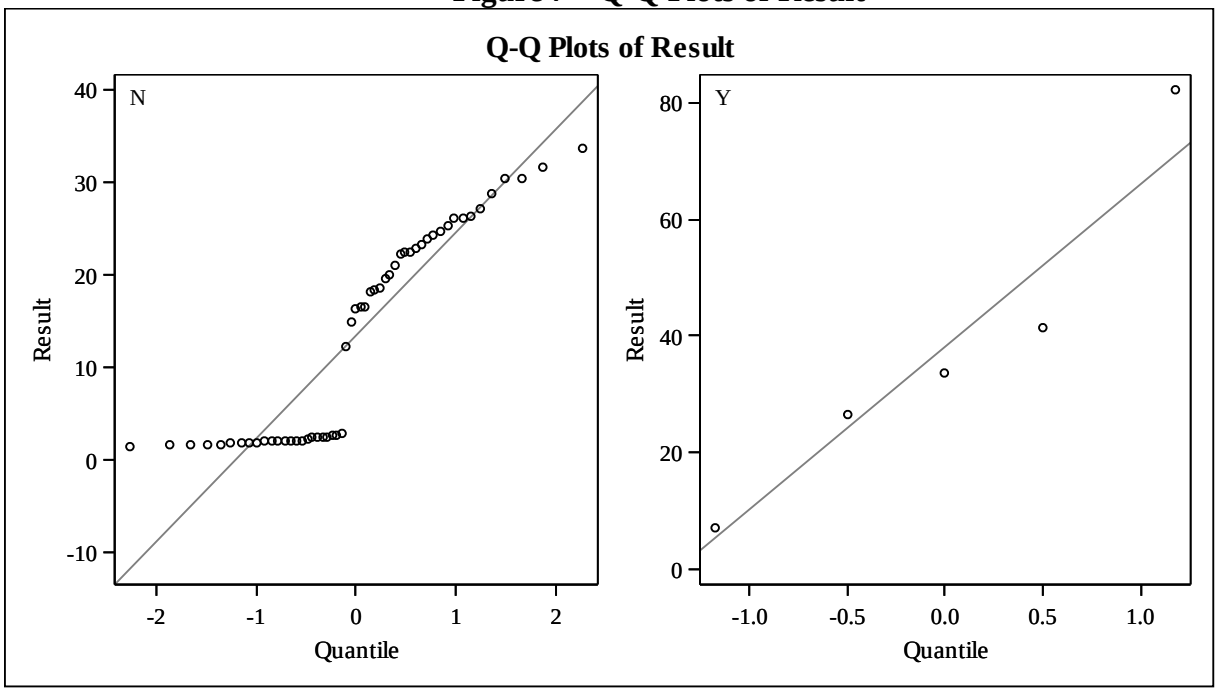
Statistical Comparison of Tails and Intestines: There are 53 readings for the tails and only five for the intestine. In the plots, the variable *intestine* is coded as Y for the cadmium in intestines and as N for the cadmium in tails. The first figure summarizes the statistical distribution for the two. Graphically, the fitted normal distribution does not fit for both tails and intestines. The figure below also depicts boxplots for these two populations. The boxplot for tails shows a long right tail and a long left tail for the intestine. This is studied further via generalized linear modeling.

Figure 6 – Distribution of Result – Tails and Intestines



A QQ plot for the tails and intestines shows mild departure from the normality. The sample size from tails is moderately large, and the central limit theorem will ensure the normality requirement for the statistical tests provided below.

Figure 7 – Q-Q Plots of Result



The comparisons of the means for the cadmium in the tails versus intestines is carried out next using the *t* test. The mean cadmium in the tails is about 4.05 µg/kg, whereas it is 16.20 in the intestine.

Table 26 – *T* Test

Intestine Type	N	Mean	Std Dev	Std Err	Minimum	Maximum
N	53	4.05	3.35	0.46	0.44	10.10
Y	5	16.20	8.83	3.95	7.20	26.60
Diff (1 – 2)		-12.15	4.00	1.87		

The table below provides 95% confidence limits for the mean cadmium contents in the tails, intestines, and the difference between the intestines and the tails. The table also provides 95% confidence limits for the standard deviations. For the mean differences, both pooled which assumes equal variances for tails and intestines, and Satterthwaite are provided in the table below. The confidence limits for the standard deviations are of the equal-tailed variety. Note that the sample standard deviation for the cadmium in the tails is 3.35 µg/kg, whereas for intestines it is 8.83 µg/kg. This difference in standard deviations (or variances) is tested via a folded f-test and the test concludes that the variances are unequal with *p* value of 0.0003.

Table 27 – 95% Confidence Limits

Type	Method	Mean	95% CL Mean		Std Dev	95% CL Std Dev	
N		4.05	3.13	4.97	3.35	2.81	4.15
Y		16.20	5.24	27.16	8.83	5.29	25.37
Diff (1 – 2)	Pooled	-12.15	-15.90	-8.40	4.00	3.38	4.91
Diff (1 – 2)	Satterthwaite	-12.15	-23.07	-1.23			

The tables below provide the *p* values for the difference in the mean cadmium contents between tails and intestines under both equal and unequal variances. Assuming the variances are equal the *p* value of the difference is highly significant (*p* value < 0.0001), whereas assuming that the variances are unequal, the *p* value is 0.0365.

Table 28 – *P* values

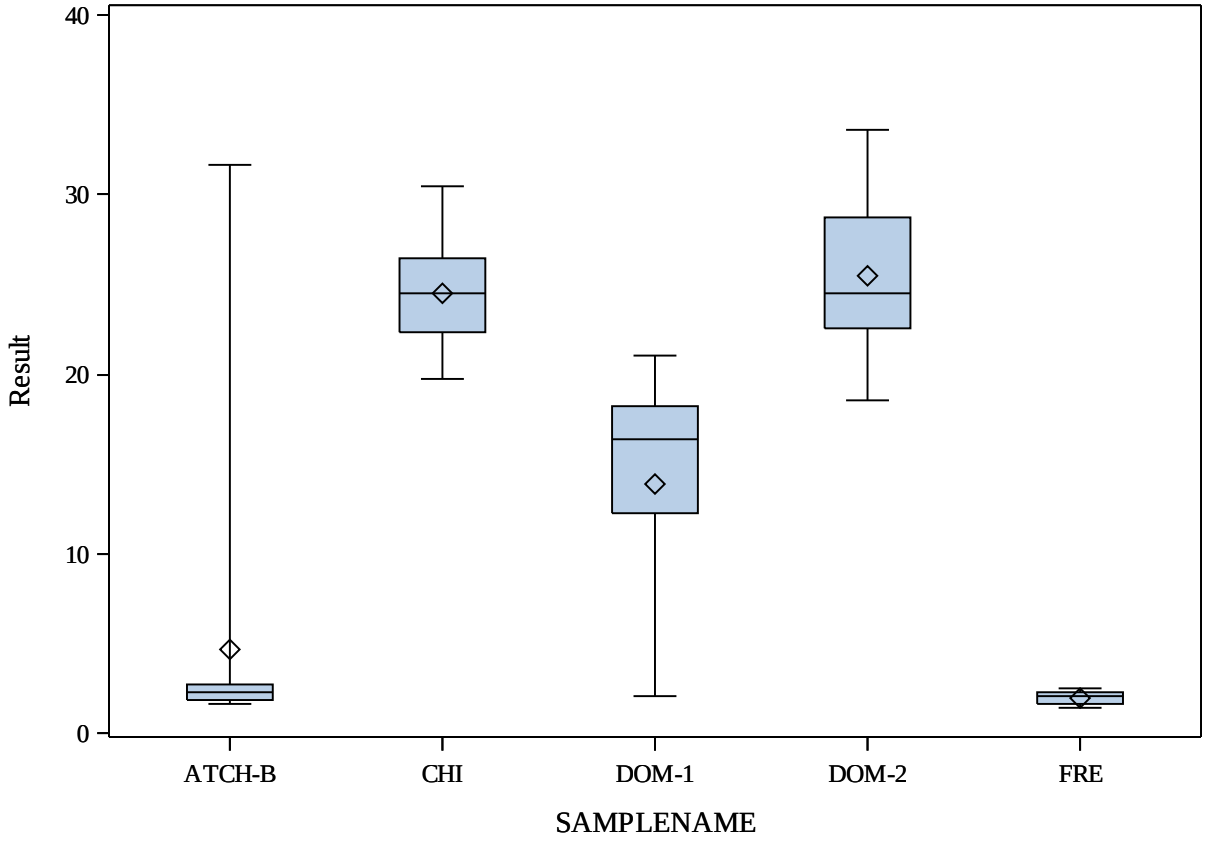
Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	56.0000	-6.49	<0.0001
Satterthwaite	Unequal	4.1094	-3.06	0.0365

Table 29 – P-value – Equality of Variances

Method	Num DF	Den DF	F Value	Pr > F
Folded F	4	52	6.94	0.0003

Statistical Comparison of Tails: The figure below shows the boxplots of cadmium contents in the crayfish tails by site.

Figure 8 – Distribution of Results – Tails



A quick visual inspection shows that ATCH-B and FRE sites have the lowest levels of cadmium in the tails, whereas DOM-2 is the highest. DOM-1 and CHI are a bit less in the cadmium contents in the tails. Next, via a GLM, an unbalanced ANOVA model is fitted.

First, we test the assumption of the equality of the variances by Levene's test. Note that the Levene's Test does not reject the assumption of homogeneity of variance with a *p* value of 0.0721.

Table 30 – Levene's Test for Homogeneity of Result Variance
ANOVA of Absolute Deviations from Group Means

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Sample Name	4	13.6204	3.4051	2.30	0.0721
Error	48	70.9991	1.4791		

Next, we report the results of the ANOVA test which assumes equality of the variances.

Table 31 – ANOVA Test Result

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	452.2500	113.0625	41.18	<0.0001
Error	48	131.7807	2.7454		
Corrected Total	52	584.0308			

The ANOVA test concludes that there is a statistically significant difference in the mean cadmium in tails from the five sites with a *p* value of <0.0001.

Tukey's Studentized Range (HSD) Test (Post Hoc): The post hoc tests are designed for further investigation into the situations when the f-test is significant and the researcher is interested in which means are significantly different from each other. The post hoc test we used is Tukey's studentized range test, which corrects for multiple tests so that a correct Type I experiment error rate is maintained even though multiple tests or comparisons are computed.

The tables below summarize the Tukey's test. Statistically significant differences at the 5% level are indicated below.

Table 32 – Turkey's Studentized Range (HSD) Test

Alpha	0.05000
Error Degrees of Freedom	48.00000
Error Mean Square	2.74540
Critical Value of Studentized Range	4.00812

Table 33 – Turkey's Studentized Range (HSD) Test

Comparisons significant at the 0.05 level are indicated by ***				
Sample Name Comparison	**Difference Between Means**	**Simultaneous 95% Confidence Limits**		
DOM-2 – CHI	0.2950	-1.8051	2.3951	
DOM-2 – DOM-1	3.4850	1.3849	5.5851	***
DOM-2 – ATCH-B	6.2281	4.2174	8.2388	***
DOM-2 – FRE	7.0433	4.9914	9.0951	***
CHI – DOM-2	-0.2950	-2.3951	1.8051	
CHI – DOM-1	3.1900	1.0899	5.2901	***
CHI – ATCH-B	5.9331	3.9224	7.9438	***
CHI – FRE	6.7483	4.6964	8.8001	***
DOM-1 – DOM-2	-3.4850	-5.5851	-1.3849	***
DOM-1 – CHI	-3.1900	-5.2901	-1.0899	***
DOM-1 – ATCH-B	2.7431	0.7324	4.7538	***
DOM-1 – FRE	3.5583	1.5064	5.6101	***
ATCH-B – DOM-2	-6.2281	-8.2388	-4.2174	***
ATCH-B – CHI	-5.9331	-7.9438	-3.9224	***
ATCH-B – DOM-1	-2.7431	-4.7538	-0.7324	***
ATCH-B – FRE	0.8152	-1.1450	2.7754	
FRE – DOM-2	-7.0433	-9.0951	-4.9914	***
FRE – CHI	-6.7483	-8.8001	-4.6964	***
FRE – DOM-1	-3.5583	-5.6101	-1.5064	***
FRE – ATCH-B	-0.8152	-2.7754	1.1450	

Summary and Limitations: The difference between tails and intestines is mildly statistically significant. Due to small sample size for intestines in the CHI, DOM-1 and DOM-2, sites having more cadmium in the tails, the overall difference between tails and intestines is mildly statistically significant

There is a strong statistically significant difference between the 5 sites. The cadmium content FRE and ATCH-B is not statistically significant. Also, DOM-2 and CHI are not significantly significant. The rest all are statistically different.

Chapter 7.7 – Copper Test Results and Statistics – Tail with Intestine

Table 34 – Tail with Intestine

Sample Name	Result (µg/kg)	Detection Limit	Reference Limit	Dilution
ATCH-B-1	286.00	1.730	2.89	1
ATCH-B-2	435.00	1.300	2.17	1
ATCH-B-3	467.00	1.110	1.84	1
ATCH-B-4	580.00	1.260	2.11	1
ATCH-B-5	371.00	1.120	1.86	1
ATCH-B-6	598.00	1.500	2.49	1
ATCH-B-7	396.00	0.976	1.63	1
ATCH-B-8	463.00	1.590	2.66	1
ATCH-B-9	598.00	1.460	2.43	1
ATCH-B-10	435.00	1.110	1.85	1
ATCH Extra	365.00	1.900	3.17	1
ATCH Extra & 11 TS	252.00	1.610	2.69	1
CHI-1	461.00	14.600	24.30	10
CHI-2	540.00	14.000	23.30	10
CHI-3	573.00	15.700	26.20	10
CHI-4	254.00	16.300	27.20	10
CHI-5	633.00	12.100	20.10	10
CHI-6	361.00	13.400	22.30	10
CHI-7	365.00	18.300	30.50	10
CHI-8	203.00	14.800	24.70	10
CHI-9	312.00	15.800	26.40	10
CHI-10	512.00	11.800	19.70	10
DOM-1-1	536.00	1.230	2.05	1
DOM-1-2	<2.13	1.280	2.13	1
DOM-1-3	419.00	8.990	15.00	10
DOM-1-4	513.00	10.900	18.20	10
DOM-1-5	333.00	9.960	16.60	10
DOM-1-6	547.00	7.360	12.30	10
DOM-1-7	480.00	9.760	16.30	10
DOM-1-8	328.00	9.920	16.50	10
DOM-1-9	390.00	12.600	21.00	10
DOM-1-10	274.00	11.000	18.40	10
DOM-2-1	278.00	15.700	26.20	10
DOM-2-2	250.00	13.500	22.50	10
DOM-2-3	292.00	17.200	28.70	10
DOM-2-4	247.00	11.100	18.50	10
DOM-2-5	367.00	13.500	22.50	10
DOM-2-6	289.00	13.700	22.80	10
DOM-2-7	296.00	14.300	23.80	10
DOM-2-8	322.00	20.200	33.60	10

(Table 34 cont.)

Sample Name	Result (µg/kg)	Detection Limit	Reference Limit	Dilution
DOM-2-9	434	18.300	30.50	10
DOM-2-10	300	15.200	25.30	10
FRE-1	358	1.490	2.49	1
FRE-2	454	1.050	1.75	1
FRE-3	267	1.510	2.52	1
FRE-4	280	1.210	2.02	1
FRE-5	361	0.873	1.45	1
FRE-6	308	0.949	1.58	1
FRE-7	319	0.986	1.64	1
FRE-8	270	1.230	2.05	1
FRE-9	460	1.100	1.84	1
FRE-10	372	1.250	2.09	1
FRE-Extra	431	1.370	2.28	1

Chapter 7.8 – Copper Test Results and Statistics – Intestine

Table 35 – Intestine

Sample Name	Result (µg/kg)	Detection Limit	Reference Limit	Dilution
ATCH-B	695	49.30	82.20	1
CHI	1680	3.74	6.240	1
DOM-1	871	20.20	33.60	1
DOM-2	289	24.90	41.50	1
FRE	589	14.90	24.80	1

Chapter 7.9 – Zinc Test Results and Statistics – Tail with Intestine

Table 36 – Tail with Intestine

Sample Name	Result (µg/kg)	Detection Limit	Reference Limit	Dilution
ATCH-B-1	7120	27.1	231	1
ATCH-B-2	8630	203.0	1730	10
ATCH-B-3	9590	173.0	1470	10
ATCH-B-4	11300	198.0	1690	10
ATCH-B-5	5680	175.0	1490	10
ATCH-B-6	11800	234.0	1990	10
ATCH-B-7	9370	153.0	1300	10
ATCH-B-8	8800	250.0	2130	10
ATCH-B-9	7440	229.0	1950	10
ATCH-B-10	8410	174.0	1480	10
ATCH Extra	9810	297.0	2530	10
ATCH Extra & 11 TS	16100	253.0	2150	10
CHI-1	8770	228.0	1940	10
CHI-2	10700	219.0	1860	10
CHI-3	8050	246.0	2090	10
CHI-4	8400	256.0	2180	10
CHI-5	14200	189.0	1610	10
CHI-6	10400	209.0	1780	10
CHI-7	10700	287.0	2440	10
CHI-8	7540	232.0	1970	10
CHI-9	8820	248.0	2110	10
CHI-10	11600	185.0	1570	10
DOM-1-1	13200	192.0	1640	10
DOM-1-2	14000	200.0	1700	10
DOM-1-3	8520	141.0	1200	10
DOM-1-4	8970	171.0	1460	10
DOM-1-5	9890	156.0	1330	10
DOM-1-6	10300	115.0	982	10
DOM-1-7	9430	153.0	1300	10
DOM-1-8	8760	155.0	1320	10
DOM-1-9	11300	197.0	1680	10
DOM-1-10	8520	173.0	1470	10
DOM-2-1	8280	246.0	2090	10
DOM-2-2	8790	212.0	1800	10
DOM-2-3	8730	270.0	2300	10
DOM-2-4	9840	173.0	1480	10
DOM-2-5	7660	211.0	1800	10
DOM-2-6	7310	214.0	1820	10
DOM-2-7	8130	223.0	1900	10
DOM-2-8	8530	316.0	2690	10

(Table 36 cont.)

Sample Name	Result (µg/kg)	Detection Limit	Reference Limit	Dilution
DOM-2-9	7560	287	2440	10
DOM-2-10	8430	238	2020	10
FRE-1	10200	234	1990	10
FRE-2	10300	164	1400	10
FRE-3	10200	237	2010	10
FRE-4	10900	190	1610	10
FRE-5	11600	137	1160	10
FRE-6	8810	148	1260	10
FRE-7	9880	154	1310	10
FRE-8	10400	192	1640	10
FRE-9	10100	173	1470	10
FRE-10	12400	196	1670	10
FRE-Extra	11700	214	1830	10

Chapter 7.10 – Zinc Test Results and Statistics – Intestine

Table 37 – Intestine

Sample Name	Result (µg/kg)	Detection Limit	Reference Limit	Dilution
ATCH-B	3750	390.0	3320	1
CHI	5910	316.0	2960	1
DOM-1	<6580	772.0	6580	1
DOM-2	4960	232.0	1980	1
FRE	7710	58.6	499	1

Chapter 8 – Discussion

The results from the four man-made ponds vary greatly from the data taken at the Atchafalaya Basin site. The mean sample data for the crawfish ponds DOM-1, DOM-2, and FRE (Tables 10 and 11) show statistical parity. The CHI samples showed a much higher arsenic, concentration compared to the Atchafalaya Basin samples, which contained the lowest levels (Table 8). Research by Luckette and LSU A & M College showed a statistically higher percentage of pancreatic cancer from cadmium in the CHI sample location as compared to the other locales (Table 21). This could be the result of arsenic and cadmium concentrations in grain fed animals (especially pork) and the amount of arsenic and cadmium-laden rice consumed there. Research suggests that 1 in 16 cancer deaths are attributable to cadmium exposure and the resultant pancreatic cancer in the CHI area (Luckett et al., 2012). The data represented in Tables 21 and 22 support this theory.

1.6 million tons of arsenic-laden fertilizers, pesticides, and insecticides have been used in Louisiana agriculture since 1901, making rice products a major source of environmental arsenic. It accumulates in plants and the effects are passed on to humans when ingested (Snyder & Slaton, 2001). Research on rice products states that MMA and DMA metabolites produced after rice is ingested may cause cancer in humans. Threshold levels of arsenic are created by continual ingestion of rice products, possibly altering DNA which can lead to mutations in the human body and cancer (Shen et al., 2007).

Water supplies are tested and monitored by municipalities and counties for MRLs by the EPA. Regulating water sources mitigates exogenous arsenic. Accumulation increases threshold levels thereby affecting DNA that may lead to cancer. (ATSDR, 2009) The natural production of crayfish in the Atchafalaya Basin seems to be less influenced by contamination from agricultural products and oil well leakages. Lower arsenic and cadmium concentration test results in the

Atchafalaya Basin show, relative to the mean value of 84 µg/kg, a lesser segregated mean percentage (Table 10). Levee protection from potentially harmful substances prevents destruction of flora and fauna, and limited use of fertilizers, pesticides, and herbicides protect indigenous plants and wildlife.

Effects from nearby sugar cane agriculture are of little consequence relative to arsenic concentrations as shown in test results in the DOM-1 and DOM-2 pond sites (Table 8). There was no indication of deleterious effects to animals in the tested areas.

Intestine samples showed a much higher concentration of arsenic as compared to tail samples (Table 9). The concentrations on average were 6-7 times higher percentages in parts per billion (ppb). The research would suggest removing the intestine after boiling before consuming the crayfish to reduce the potentially harmful effects of inorganic arsenic (Table 9). It is unclear whether ingestion of organic arsenic in crayfish contributes to a risk of cancer, and more research is needed.

Copper is an essential nutrient for homeostasis in the human body. Food sources usually supply the body with ample amounts. The RDA for copper is 0.9 mg/day, whereas the median intake from a typical U.S. diet ranges from 1-1.6 mg/day. The safest highest intake of copper for chronic exposure is 10 mg/day. Copper intake in the tested areas is substantially higher than daily tolerable intake values. Ingesting 20-25 crayfish in one meal is enough to surpass the tolerable intake level of 10,000 ppb. Copper concentrations are somewhat greater in the crayfish intestine, but not so in all samples tested (Tables 34 and 35). The DOM-2 pond showed an appreciable lower difference in the copper concentration, but more research is needed to quantify better statistics. If the tails were consumed without the intestines, copper concentrations could be reduced.

Zinc concentration results may also surpass the minimum or adequate levels for dietary consumption. The upper allowable intake of zinc is 40 mg/day. The crayfish tails tested depict higher concentrations than the UL and pose the risk of deleterious effects to humans or animals. The research showed that the intestine zinc levels were lower compared to the other 3 metals tested (Tables 36 and 37).

Chapter 9 – Conclusion

The WHO has established .25 mg/kg as the average daily food and as much as 2.4 – 16.7 mg/kg in seafood. Water guidelines for oral ingestion by municipalities set by the EPA is 10 µg/L (WHO, 2001). Arsenic intake differs with foods ingested. Rice contains a higher level of inorganic arsenic obtained from pesticides, herbicides, fungicides, and fertilizers used in the growing process. Most foods would be contaminated with pesticides or herbicides to some degree, unless grown with no application for eradication or control of insects and weeds. Cereals and grains have a higher content of cadmium as well as tobacco, along with leafy vegetables such as lettuce (Roman et al., 2010).

The research herein suggests the median content for arsenic in the ponds and Atchafalaya Basin wild domain tested is 84 µg/k per tail sample, inclusive of the intestine. There is a higher percentage of arsenic in the CHI samples tested in tail tissue, as compared to the other 3 man-made ponds, and a lower arsenic content in the Atchafalaya Basin samples. The research showed the arsenic content in the Atchafalaya Basin crayfish at a lower concentration than all samples tested as the result of little or no effect from pesticides, herbicides, and fungicides.

Research herein shows a much higher concentration of arsenic in the intestine, 6-7 times higher, suggesting that one should not consume the intestine when eating crayfish. There is no conclusive research that crayfish themselves cause disease. Based on these results, the possible accumulation of arsenic in crayfish, depending incidence and amounts consumed annually could be responsible for deleterious effects, such as DNA interruption and even cancer. Consuming the tail and intestine is the basis for arsenic, cadmium, copper, and zinc concentrations possibly surpassing the MRL guidelines as set by the ATSDR and other government agencies.

Future research projects should also include eastern Louisiana as well as southeast Texas, and central to southern Arkansas. This would provide a more pervasive statistical analysis of heavy metal percentages from the three states that produce high volumes of crayfish for consumption. Soil samples and water supplies would also be addressed in each venue, before commencing with tail and intestine testing.

Differences in metal concentrations could possibly be traced back to the indigenous metal nomenclature of the soil or usage of a product from a particular pesticide, herbicide, fungicide or fertilizer. The testing of tail tissues with the intestine removed would also provide further analysis of heavy metal contamination.

Bibliography

Adhami, E. J., & Cullufi, P. (1995, March). Wilson's disease in Albania. *Panminerva Medica, 34*:1, 18-21.

Agency for Toxic Substances and Disease Registry. (2003). Health effects. 21-117. Retrieved from http://www.atsdr.cdc.gov/toxprofiles/tp60-c3.pdf

Agency for Toxic Substances and Disease Registry. (2003, September). Toxicological profile for malathion. Retrieved from http://www.atsdr.cdc.gov/toxprofiles/tp154.pdf

Agency for Toxic Substances and Disease Registry. (2005, August). Toxguide for zinc. Retrieved from http://www.atsdr.cdc.gov/toxguides/toxguide-60.pdf

Agency for Toxic Substances and Disease Registry. (2005). Toxicological profile for zinc. Retrieved from http://www.atsdr.cdc.gov/toxprofiles/tp60.pdf

Agency for Toxic Substances and Disease Registry. (2006). Potential for human exposure. 313-381. Retrieved from http://www.atsdr.cdc.gov/toxprofiles/tp2-c6.pdf

Agency for Toxic Substances and Disease Registry. (2007). Regulations and advisories. 395-399. Retrieved from http://www.atsdr.cdc.gov/toxprofiles/tp2-c8.pdf

Agency for Toxic Substances and Disease Registry. (2007, August). Toxicological profile for arsenic. Retrieved from http://www.atsdr.cdc.gov/toxprofiles/tp2.pdf

Agency for Toxic Substances and Disease Registry. (2009). Case studies in environmental medicine. 1-124. Retrieved from www.atsdr.cdc.gov/csem/arsenic/docs/arsenic.pdf

Agency for Toxic Substances and Disease Registry. (2012). Potential for human exposure. 277-331. Retrieved from http://www.atsdr.cdc.gov/toxprofiles/tp5-c6.pdf

Agency for Toxic Substances and Disease Registry. (2012, October). Toxguide for cadmium. Retrieved from http://www.atsdr.cdc.gov/toxguides/toxguide-5.pdf

Agency for Toxic Substances and Disease Registry. (2015, September). Minimum risk levels (MRLs) list. Retrieved from http://www.atsdr.cdc.gov/mrls/mrllist.asp

Anderson, M. B., Reddy, P., Preslan, J. E., Fingerman, M., Bollinger, J., Jolibois, L., …George, W. J. (1997, April). Metal accumulation in crayfish, Procambarus clarkii, exposed to a petroleum-contaminated bayou in Louisiana. *Ecotoxicology and Environmental Safety*, *37*, 267-272.

Angenard, G., Muczynski, V., Coffigny, H., Pairault, C., Duquenne, C., Frydman, R., …Livera, G. (2010, March). Cadmium increases human fetal germ cell apoptosis. *Environmental Health Perspectives*, *118*:3, 331-337. doi:10.11289/ehp.0900975

Avan, A., & Hoogenraad, T. U., (2015, May). Zinc and copper in Alzheimer's disease. *Journal of Alzheimer's Disease*, *46*:1, 89-92. doi:10.3233/jad-150186

Avery, J., & Lorio, W. (1999, July) Crawfish production manual, pub 2637. 11-38. Retrieved from http://www.agctr.lsu.edu/wwwac

Bandmann, O., Weiss, K. H., & Kaler, S. G. (2015, January). Wilson's disease and other neurological copper disorders. *The Lancet Neurology*, *14*:11, 103-113.

Becker, D., & Mergel, M. (2010, July). Permethrin. Retrieved from http://www.toxipedia.org/display/toxipedia/permethrin

Bellows, B. C. (2005). Arsenic in poultry litter: organic regulations. *ATTRA, The National Sustainable Agriculture Information Service*, 1-11.

Benoff, S., Jacob, A., & Hurley I. R. (2000). Male infertility and environmental exposure to lead and cadmium. *Human Reproduction Update*, *6*:2, 197-121.

Beyersmann, D., & Hartwig, A. (2008, April). Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms. *Archives of Toxicology*, *18*:8, 493-512.

Brown, K. H. (1998, August). Effect of infections on plasma zinc concentration and implications for zinc status assessment in low-income counties. *The American Journal of Clinical Nutrition*, *68*, supp. 2, 425S-429S.

Calabrese, E. J., & Baldwin, L. A. (2001, May). U-shaped dose-responses in biology, toxicology, and public health. *Annual Review of Public Health*, *22*, 15-33.

Chakraborti, D. (2001). Fate of three crore repee arsenic removal plants in Murshidabad. School of Environmental Studies, Jadavpur U. Retrieved from http://www.sustainablefuture.se/arsenic/docs/chakraborti-murshidad.pdf

Cho, Y. A., Kim, J., Woo, H. D., & Kang, M. (2013, September). Dietary cadmium intake and the risk of cancer: a meta-analysis. *Plos One*, *8*:9. doi:10.1371/journal.pone.0075087

Choi, B. S., Choi, S. J., Kim, D. W., Huang, M., Kim, N. Y., Park, K. S., …Park, J. D. (2010, January), Effects of repeated seafood consumption on urinary excretion of arsenic species by volenteers. *Archives of Environmental Contamination Toxicology*, *58*:1, 222-229.

Choudhary, S. V., Gadegone, R. W., & Koley, S. (2012, September – October). Menkes kinky hair disease. *Indian Journal of Dermatology*, *57*:5, 407-409. doi:10.4103/0019-5154.100503

Codex Alimentarius Commission. (2013, February). Joint FAO/WHO food standards programme codex committee on contaminants in foods. Retrieved from ftp://ftp.fao.org/codex/reports/reports_2014/rep14_cfe.pdf

Consumer Reports. (2012, November). Arsenic in your food. 22-28. Retrieved from http://www.consumerreports.org/cro/magazine/2012/11/arsenic-in-your-food/index.htm

Contreras-Acuna, M., Garcia-Barrera, T., Garcia-Sevillano, M. A., & Gomez-Ariza, J. L. (2013) Speciation of arsenic in marine food (anemonia sulcata) by liquid chromatography coupled to inductivity coupled plasma mass spectrometry and organic mass spectrometry. *Journal of Chromatography A*, 133-144. doi:http://dx.doi.org/10.1016/j.chroma.2013.01.068

Cubadda, F., Aureli, F., D'Amato, M., Raggi, A., Turco, A. C., & Mantovani, A. (2012, January). Speciated urinary arsenic as a biomarker of dietary exposure to inorganic arsenic in residents living in high-arsenic areas in Latium Italy. *Pure and Applied Chemistry*, *84*:2, 203-214. doi:http://dx.doi.org/10.1351/PAC-CON-11-09-29

Cubadda, F., D'Amato, M., Mancini, F. R., Aureli, F., Raggi, A., Busani, L., & Mantovani, A. (2015, January). Assessing human exposure to inorganic arsenic in high-arsenic areas of latium: a biomonitoring study integrated with indicators of dietary intake. *Annali di igiene: medicina preventiva e di cinybuta*, *27*:1, 39-51. doi:10.7416/ai.2015.2021

Cui, X., Kobayashi, Y., Hayakawa, T., & Hirano, S. (2004, August). Arsenic speciation in bile and urine following oral and intravenous exposure to inorganic and organic arsenics in rats. *Toxicological Sciences*, *82*, 478-487. doi:10.1093/toxsci/kfh265

Dauphine, D. C., Ferreccio, C., Guntur, S., Yuan, Y., Hammond, S. K., Balmes, J., …Steinmaus, C. (2011, August). Lung function in adult following in utero and childhood exposure to arsenic in drinking water: preliminary findings. *International Archives of Occupational and Environmental Health*, *84*:6, 591-600. doi: 10.1007/s00420-010-0591-6

Davies, N.T. (1980). Studies on the absorption of zinc by rat intestine. *British Journal of Nutrition*, *43*, 189-203.

Davis, M. A., Mackenzie, T. A., Cottingham, K. L., Gilbert-Diamond, D., Punshon, T., & Karagas, M. R. (2012, October). Rice consumption and urinary arsenic concentrations in U.S. children. *Environmental Health Perspectives, 120*:10, 1418-1424. doi:10.1289/ehp.1205014

Department of Environmental Quality. (2000). Arsenic sampling results explained. 29-30. Retrieved from http://www.deq.louisiana.gov/portal/portals/0/news/pdf/arsenicexplainedjan10.pdf

Devesa, V., Loos, A., Suner, M. A., Velez, D., Feria, A., Martínez, A., …Sanz, Y. (2005, December). Transformation of organoarsenical species by the microflora of freshwater crayfish. *Journal of Agricultural and Food Chemistry, 53,* 10297-10305. doi:10.1021/jf050423q

Dijkstra, M., Veld, G. I., van den Berg, G. J., Muller, M., Kuipers, F., & Vonk, R. J. (1995, January). Adenosine triphosphate-dependent copper transport in isolated rat liver plasma membranes. *The Journal of Clinical Investigations, 95*:1, 412-416. doi:10.1172/jci117670

Durham University. (2010, October). Arsenic resistance mechanisms. Retrieved from https://www.dur.ac.uk/cid/research/arsenic_resistance_mechanisms/

Duxbury, J. M., & Zavala, Y. J. (2012). What are safe levels of arsenic in food and soils? Retrieved from http://arsenic.tamu.edu/pub/pubpres/DHAKA/dhaka3.pdf

Environmental Protection Agency. Arsenic Rule. Retrieved from http://water.epa.gov/lawsregs/rulesregs/sdwa/arsenic/regulations.cfm

Ferenci, P., Litwin, T., Seniow, J., & Czlonkowska, A. (2015, March). Encephalopathy in Wilson's disease: copper toxicity or liver failure? *Journal of Clinical and Experimental Hepatology, 5*, supp. 1, S88-S95. doi:http://dx.doi.org/10.1016/j.jceh.2014.09.002

Ferm, V. H. (1997, August). Arsenic as a teratogenic agent. *Environmental Health Perspectives*, *19*, 215-217.

Finerty, M. W., Madden, J. D., Feagley, S. E., & Grodner, R.M. (1990). Effect of environs and seasonality on metal resides in tissues of wild and pond-raised crayfish in southern Louisiana. *Archives of Environmental Contamination and Toxicology*, *19*, 94-100.

Food and Drug Administration. (2011). Questions and answers regarding 3-nitro (roxarsone). Retrieved from www.fda.gov/animalveterinary/safetyhealth/productsafetyinformation/ucm258313.htm

Food and Drug Administration. (2015, April). FDA announces pending withdrawal of approval of nitarsone. Retrieved from http://www.fda.gov/animalveterinary/newsevents/cvmupdates/ucm440668.htm

Food and Drug Administration. (2015, April). Title 21--food and drugs. *2.* Retrieved from http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=165.110

Fosmire, G. J. (1990). Zinc toxicity. *The American Journal of Clinical Nutrition*, *51*, 225-227.

Gianessi, L. (2009, March). The benefits of insecticide use: sugarcane. *Croplife Foundation, Crop Protection Research Institute*. Retrieved from https://croplifefoundation.files.wordpress.com/2012/07/combined_document_sugarcane.pdf

Green, R. M., & Abdelghani, A. A. (2004, September). Effects of long-term exposure of the red swamp crawfish Procambarus clarkii to a mixture of two herbicides, 2,4-dichlorophenoxyacetic acid and monosodium methanearsonate, and associated human health risks. *International Journal of Environmental Research and Public Health*, *1*:2, 124-131.

Gupta, A., & Chandra, S. V. (1991). Gestational cadmium exposure and brain development: a biochemical study. *Industrial Health, 29*:2, 65-71.

Hall, M., Gamble, M., Slavkovich, V., Liu, X., Levy, D., Cheng, Z., & Graziano, J. (2007, October). Determinants of arsenic metabolism: blood arsenic metabolites, plasma folate, cobalamin, and homocysteine concentrations in maternal-newborn pairs. *Environmental Health Perspectives, 115*:10, 1503-1509.

Haliasos, E. C., Litwack, P., Kristal, L., & Chawla, A. (2007, June). Acquired zinc deficiency in full-term newborns from decreased zinc content in breast milk. *79*, 425-428.

Hambridge, M. (2000, May). Human zinc deficiency. *The Journal of Nutrition, 130*:5, 1344S-1349S.

Hasegawa, H., Sohrin, Y., Seki, K., Sato, M., Norisuye, K., Naito, K., …Matsui, M. (2001, April). Biosynthesis and release of methylarsenic compounds during the growth of freshwater algae. *Chemosphere, 43*:3, 265-272.

Henson, M. C., & Chedrese, P. J. (2004). Endocrine disruption by cadmium, a common environmental toxicant with paradoxical effects on reproduction. experimental biology and medicine. *229*, 383-392.

Hoogenraad, T, U., (2011, September). Paradigm shift in treatment of Alzheimer's disease: zinc therapy now a conscientious choice for care of individual patients. *International Journal of Alzheimer's Disease, 2011*, 1-6. doi:org/10.4061/2011/492686

International Agency for Research on Cancer. (1991). Occupational exposures in insecticide application, and some pesticides. *IARC monographs on the evaluation of carcinogenic risk to humans,53*. Retrieved from http://monographs.iarc.fr/ENG/Monographs/vol53/mono53.pdf

International Agency for Research on Cancer. (2012). Arsenic, metals, fibers, and dusts. *IARC monographs, 100C*. Retrieved from http://monographs.iarc.fr/ENG/Monographs/vol100C/mono100C.pdf

Johnson, M. D., Kenney, N., Stoica, A., Hilakivi-Clarke, L., Singh, B., Chepko, G. …Martin, M. B. (2003, August). Cadmium mimics the in vivo effects of estrogen in the uterus and mammary gland. *Nature Medicine*. *9*:8, 1081-1084.

Kaler, S. G. (2011, January). ATP7A-related copper transport diseases-emerging concepts and future trends. *Nature Reviews, Neurology*, *7*:1, 15-29. doi:10.1038/nmeurol.2010.180

Kaur, S., K., Kamli, M. R., & Ali, A. (2011, October). Role of arsenic and its resistance in nature. *Canadian Journal of Microbiology*, *57*, 769-774. doi:10.1139/W11-062

Kile, M. L., Baccarelli, A., Hoffman, E., Tarantini, L., Quamruzzaman, Q., Rahman, M., …Christiani, D. C. (2012, July). Prenatal arsenic exposure and DNA methylation in maternal and umbilical cord blood leukocytes. *Environmental Health Perspectives*, *120*:7, 1061-1066.

Kumar, N. (2006, October). Copper deficiency myelopathy (human swayback). *Mayo Clinic Proceedings*, *81*:10, 1371-1384.

Lazarevic, K., Nikolic, D., Stosic, L., Milutinovic, S., Videnovic, J., & Bogdanovic, D. (2012, March). Determination of lead and arsenic in tobacco and cigarettes: an important issue of public health. *Central European Journal of Public Health*, *20*:1, pp. 62-66.

Lelie H. L., Liba, A., Bourassa, M. W., Chattopadhyay, M., Chan, P. K., Gralla, E. B., …Whitelegge, J. P. (2011, January). Copper and zinc metallation status of copper-zinc superoxide dismutase from amyotrophic lateral sclerosis transgenic mice. *The Journal of Biological Chemistry*, *286*:4, 2795-2806. doi: 10.1047/jcb.M110.186999

Lin, Y., Ho, C., Hsu, C., Lin, J., Chuang, C., Tsai, J., …Lin, S. (2014, May). A truncating de novo point mutation in a young infant with severe Menkes disease. *Pediatrics and Neonatology, 2014*, 1-4. doi:org/10.1016/j.pedneo.2014.05.008

Linder, M. C., Wooten, L., Cerveza, P., Cotton, S., Shulze, R., & Lomeli, N. (1998, May). Copper transport. *The American Journal of Clinical Nutrition, 67*, supp. 5, 965S-971S.

Lokuge, K. M., Smith, W., Caldwell, B., Dear, K., & Milton, A. H. (2004, August). The effect of arsenic mitigation interventions on disease burden in Bangladesh. *Environmental Health Perspectives*, *112*:11, 1172-1177

Loudianos, G., Kostic, V., Solinas, P., Lovicu, M., Dessì, V., Svetel, M., …Cao, A. (2003, June). Characterization of the molecular defect in the ATP7B gene in Wilson's disease patients from Yugoslavia. *Genetic Testing*, *7*:2, 107-112. doi:10.1089/109065703322146786

Luckett, B. G., Su, J., Rood, J. C., & Fontham, E. T. H. (2012, November). Cadmium exposure and pancreatic cancer in south Louisiana. *Journal of Environmental and Public Health, 2012*, 1-11. doi:org/10.1155/2012/180186

Lundh, D., Larson, D., Nahar, N., & Mandal, A. (2010). Arsenic accumulation in plants – outlining strategies for developing improved variety of crops for avoiding arsenic toxicity in foods. *Journal of Biological Systems*, *18*:1, 223-241. doi: 10.1142/S0218339010003214

Mazumder, D. N. G. (2000, June). Chapter 4: diagnosis and treatment of chronic arsenic poisoning. 1-35. Retrieved from http://www.who.int/water_sanitation_health/dwq/arsenicun4.pdf

Merialdi, M., Caulfield, L. E., Zavaleta, N., Figueroa, A., & DiPietro, J. A. (1999, February). Adding zinc prenatal iron and folate tablets improves fetal neurobehavioral development. *American Journal of Obstetrics and Gynecology*, *180*:2, 483-490.

Methfesssel, A. H., & Spencer, H. (1973, February). Zinc metabolism in the rat and intestinal absorption of zinc. *Journal of Applied Physiology, 34*:1, 58-62.

Miller Jr., W. H., Schipper, H. M., Lee, J.S., Singer, J., & Waxman, S. (2002, July). Mechanisms of action of arsenic trioxide. *Cancer Research, 62,* 3893-3903. Retrieved from http://cancerres.aacrjournals.org/content/62/14/3893.full.html

Moynahan, E. J. (1974, August). Acrodermatitis enteropathica: a lethal inherited human zinc-deficiency disorder. *The Lancet, 304*:7877, 399-400.

National Academies. (2001). Dietary reference intakes: elements. *Food and Nutrition Board, Institute of Medicine, The National Academies*. Retrieved from http://iom.nationalacademies.org/~/media/files/activity%20files/nutrition/dris/dri_elements.pdf

National Academies. (2001). Dietary reference intakes (DRIs): estimated average requirements. *Food and Nutrition Board, Institute of Medicine, The National Academies*. Retrieved from http://iom.nationalacademies.org/~/media/files/activity%20files/nutrition/dris/5_summary%20table%20tables%201-4.pdf

National Academies. (2001). Dietary reference intakes (DRIs): tolerable upper intake levels, vitamins. *Food and Nutrition Board, Institute of Medicine, The National Academies*. Retrieved from http://iom.nationalacademies.org/activities/nutrition/summarydris/~/media/files/activity%20files/nutrition/dris/uls%20for%20vitamins%20and%20elements.pdf

Nations, S. P., Boyer, P. J., Love, L. A., Burritt, M. F., Butz, J. A., Wolfe, G. I, …Trivedi, J. R. (2008, August). Denture cream: an unusual source of excess zinc, leading to hypocupremia and neurologic disease. *Neurology, 71*:9, 639-643. doi:10.1212/01.wnl.0000312375.79881.94

New Hampshire Department of Environmental Services. (2013). Copper: health information summary. Retrieved from http://des.nh.gov/organization/commissioner/pip/factsheets/ard/documents/ard-ehp-9.pdf

Nriagu, J. (2007). Zinc toxicity in humans. *School of Public Health, University of Michigan*, 1-7.

Petrick, J. S., Blachere, F. M., Selmin, O., & Lantz, R.C.. (2009, May). Inorganic arsenic as a developmental toxicant: in utero exposure and alterations in the developing rat lung. *Molecular Nutrition & Food Research, 53*:5, 583-591. doi:10.1002mnfr.200800019

Prasad, A. S. (1983). Zinc deficiency in human subjects. *Progress in Clinical and Biological Research, 129*, 1-33.

Rahman, M. A., Hasegawa, H., & Lim, R. P. (2012, March). Bioaccumulation, biotransformation and trophic transfer of arsenic in the aquatic food chain. *Environmental Research, 116*, 118-135. doi:org/10.1016/j.envres.2012.03.014

Richardson, J. (2012, October). Arsenic: it's what's for dinner. *Salon*. Retrieved from www.salon.com/2012/10/08/arsenic_its_whats_for_dinner

Roman, D. A ., Pizarro, I., Rivera, L., Camara, C., Palacios, M. A., Gomez, M. M., & Solar, C. (2010). An approach to the arsenic status in cardiovascular tissues of patients with coronary heart disease. *Human and Experimental Toxicology, 30*:9, 1150-1164.

Salnikow, K., & Zhitkovich, A. (2008, January). Genetic and epigenetic mechanisms in metal carcinogenesis and co-carcinogenesis: nickel, arsenic and chromium. *Chemical Research in Toxicology*, *21*:1, 28-44. doi:10.1021/tx700198a

Samarawickrama, G. P., & Webb, M. (1979, February). Acute effects of cadmium on the pregnant rat and embryo-fetal development. *Environmental Health Perspectives*, *28*, 245-249.

Samuel, J. B., Stanley, J.A., Princess, R.A., Shanthi, P., & Sebastian, M. S. (2011, September). Gestational cadmium exposure-induced ovotoxicity delays puberty through oxidative stress and impaired steroid hormone levels. *Journal of Medical Toxicology*, *7*:3, 195- 204. doi:10.1007/s13181-011-0143-9

Schwartz, G. G., & Reis, I. M. (2000, February). Is cadmium a cause of human pancreatic cancer? *Cancer Epidemiology, Biomarkers & Prevention*, *9*, 139-145.

Shapira, S. K., Neish, A. S., & Pober, B. R. (1992, July). Unknown syndrome in sibs: Pili torti, growth delay, developmental delay, and mild neurological abnormalities. *Journal of Medical Genetics*, *29*:7, 509-510.

Shen, J., Liu, J., Xie, Y., Diwan, B. A., & Waalkes, M. P. (2007, February). Fetal onset of aberrant gene expression relevant to pulmonary carcinogenesis in lung adenocarcinoma development induced by in utero arsenic exposure. *Toxicological Sciences*, *95*:2, 313-320. doi:10.1093/toxsci/kfl151

Siu, E. R., Mruk, D. D., Porto, C. S., & Cheng, Y. (2009, August). Cadmium-induced testicular injury. *Toxicology and Applied Pharmacology*, *238*:3, 240-249. doi:10.1016/j.taap.2009.01.028

Snyder, C. S., & Slaton, N. A. (2001). Rice production in the united states – an overview. *Better Crops*, *85*:3, 3-7.

Tang, j., Robertson, S., Lem, K. E., Godwin, S. C., & Kaler, S. G. (2006, November). Functional copper transport explains neurologic sparing in occipital horn syndrome. *Genetics in Medicine*, *8*, 711-718. doi: 10.1097/01.gim.0000245578.94312.1e

Thompson, J., & Bannigan, J. (2008, April). Cadmium: toxic effects on the reproductive system and the embryo. *Reproductive Toxicology*, *25*:3, 304-315.

Tokar, E. J., Qu, W., & Waalkes, M. P. (2012, March). Renal, hepatic, pulmonary and adrenal tumors induced by prenatal inorganic arsenic followed by dimethylarsinic acid in adulthood in CD1 mice. *Toxicology Letter*, *209*:2, 179-185. doi:10.1016/j.toxlet.2011.12.016

Tunca, E., Ucuncu, E., Ozkan, A. D., Ulger, Z. E., & Tekinay, T. (2013, January). Tissue distribution and correlation profiles of heavy-metal accumulation in the freshwater crayfish Astacus leptodactylus. *Archives of Environmental Contamination and Toxicology*, *64*, 676-691. doi: 10.1007/s00244-012-9863-3

Valko, M., Morris H, Cronin M. T. (2005). Metals, toxicity and oxidative stress. *Current Medicinal Chemistry*, *12*:10, 1161-1208.

Van de Wiele, T., Gallawa, C. M., Kubachka, K. M., Creed, J. T., Basta, N., Dayton, E. A., …Bradham, K. (2010, July). Arsenic metabolism by human gut microbiota upon in vitro digestion of contaminated soils. *Environmental Health Perspectives*, *118*:7, 1004-1009.

Waalkes, M. P., Ward, J. M., Liu, J., & Diwan, B. A. (2003, January). Transplacental carcinogenicity of inorganic arsenic in the drinking water: induction of hepatic, ovarian, pulmonary, and adrenal tumors in mice. *Toxicology and Applied Pharmacology*, *186*:1, 7-17.

Wapnir, R. A. (1998, May). Copper absorption and bioavailability. *The American Journal of Clinical Nutrition, 67,* supp. 5, 1054S-1060S.

Wardhana, R., & Datau, E. A. (2014, July). Metal fume fever among galvanized welders. *Acta Medica Indonesiana – The Indonesian Journal of Internal Medicine, 46*:3, 256-261.

World Health Organization. (2001). Arsenic and arsenic compounds. *Environmental Health Criteria, 224,* 2nd ed. Retrieved from http://apps.who.int/iris/bitstream/10665/42366/1/WHO_EHC_224.pdf

World Health Organization. (2011). Arsenic in drinking-water. Retrieved from http://www.who.int/water_sanitation_health/dwq/chemicals/arsenic.pdf

Xu, C., Johnson, J. E., Singha, P. K., Jones, M. M., Yan, H., & Carter, C. E. (1996, January). In vivo studies of cadmium-induced apoptosis in testicular tissue of the rat and its modulation by a chelating agent. *Toxicology, 107*:1, 1-8. doi:10.1016/0300-483X(95)03195-L

Yoshida, F. (1999, September). Itai-itai disease and the countermeasures against cadmium pollution by the Kamioka mine. *Environmental Economics and Policy Studies, 2*:3, 215-229.

Zhao, Y., Gao, S., Chou, I., Toselli, P., Stone, P., & Li, W. (2006, January). Inhibition of the expression of lysyl oxidase and its substrates in cadmium-resistant rat fetal lung fibroblasts. *Toxicological Sciences, 90*:2, 478-489. doi:10.1093/toxsci/kfj112

Zlatic, S., Comstra, H. S., Gokhale, A., Petris, M. J., & Faundez, V. (2015, September). Molecular basis of neurodegeneration and neurodevelopmental defects in Menkes disease. *Neurobiology of Disease, 81,* 154-161. doi:10.1016/j.nbd.2014.12.024

Vita

E. Gerald "T-boy" Hebert returned to Academia in 2009 at University of Louisiana at Lafayette after a thirty-nine year hiatus. The first 4 courses were taken online at Northwestern State University. He then attended University of Louisiana at Lafayette as a part time and full time student. He earned a General Studies Degree at University of Louisiana at Lafayette, and afterwards began the Master of Science Program at UNO in Earth and Environmental Sciences. He has been a longtime businessman in the oil and gas industry. In today's world of technology, he embraces higher education as enhancement to successful living. His personal life's quest was to obtain a college degree and has done so.