南華大學科技學院永續綠色科技碩士學位學程

碩士論文

Master Program of Green Technology for Sustainability
College of Science and Technology
Nanhua University
Master Thesis

有機及慣型農法對葉菜類硝酸鹽濃度影響及對台灣居民之健康風險評估

Assessing Human Health Risk on Nitrate Content in Leafy Vegetables Grown under Organic and Conventional Farming in Taiwan

瑪莉琳

Marilyn Bariga Aldamar

指導教授：陳柏青 博士

Advisor: Bo-Ching Chen, Ph.D.

中華民國 111 年 6 月

June 2022

南 華 大 學

永續綠色科技碩士學位學程

碩 士 學 位 論 文

有機及慣型農法對葉菜類硝酸鹽濃度影響及對台灣居民之健康風險評估

Assessing Human Health Risk on Nitrate Content in Leafy Vegetables Grown under Organic and Conventional Farming in Taiwan

研究生：瑪莉琳 ALDAMAR, MARILYN BARIGA

經考試合格特此證明

口試委員：劉程偉
淩燿明
陳柏青

指導教授：陳柏青

系主任(所長)：淩燿明

口試日期：中華民國 111 年 6 月 23 日

ACKNOWLEDGEMENT

I would like to express my deepest gratitude to our advisor, Professor Chen Bo-Ching. The assistance, guidance, and unwavering patience we received from him throughout the entire thesis process have been greatly appreciated. I am certain that I could not have carried out this research without his assistance.

Professor Hong Yao-Ming is also to be thanked for without him, I could not have undertaken this journey. He had contributed so much knowledge to us, especially during our thesis defense. I’ll always be thankful for his kindness and unending encouragement.

My gratitude also extends to Professor Liu Cheng-Wei for serving as one of our committee members. He was kind enough to lend us some of his time and to share his data with us. This study would not have been possible without the resources he provided.

I am always grateful for the love and encouragement I received from my family. Their emotional and spiritual support helped me greatly during my entire journey here. No matter where I am on this journey, I always know I am not alone, even if I am away from home. Moreover, I would like to thank my friends for their motivations and their presence throughout the process. It was because of them that I was able to get through the hard times. I am especially grateful to Cheska and Chrecel who have been with me throughout this journey. I feel blessed to have you guys in my life. May we continue to walk on the flower path together.

To Bangtan Sonyeondan, thank you. It's your music and words that motivate me to keep fighting even on my darkest days. You guys are one of the reasons why I made it this far.

And above all else, I want to give the highest glory and thanksgiving to my greatest confidant, our Almighty Father. I wouldn't be able to overcome all the hardships and silent battles if not for the strength that He has given me. All glory belongs to Him.

Author,

Marilyn Bariga Aldamar

摘要

蔬菜在種植過程中會累積大量的硝酸鹽，尤其是葉菜類蔬菜。由於植物葉子需要氮才能進行光合作用，使得大量的硝酸鹽累積在葉和莖組織中，根部亦會有少量累積。另一方面，由於蔬菜具有許多健康上的益處，因此為人類飲食不可或缺的一部分，也因此蔬菜被廣泛認為是人體攝取硝酸鹽的重要來源，而飲食中的硝酸鹽與高鐵血紅蛋白血症和胃癌等致命疾病的風險增加有關。因此，本研究調查了臺灣普遍種植及最常被食用的八種不同蔬菜的硝酸鹽含量，比較有機和慣行農法栽培蔬菜的硝酸鹽濃度，以探討不同耕作方式對硝酸鹽的累積濃度的影響；此外，本研究亦基於毒理學，計算台灣地區成年男性和女性，因攝食蔬菜而暴露於硝酸鹽之風險。研究中採用美國環境保護署（US EPA）發布之人體健康風險評估準則，評估臺灣居民的致癌及非致癌風險。為考量參數之不確定性，將參數以機率分布表示，並使用 Oracle Crystal Ball 軟體執行蒙地卡羅模擬法。

研究結果顯示，有機蔬菜硝酸鹽濃度的幾何平均值和幾何標準偏差值分別為 996.02 mg/kg 和 4.37，慣行蔬菜則為 2497.97 mg/kg 和 1.91(慣行蔬菜之樣品數為 192、有機蔬菜之樣本數為 38)。在非致癌風險部分，研究結果顯示男性和女性於攝食慣行栽培蔬菜時，危害商數 HQ 之平均值分別為 0.23 和 0.28；若攝食有機蔬菜，則男性及女性之 HQ 值分別為 0.30 及 0.36，代表不論攝食慣行或有機蔬菜，非致癌風險為可接受的。若保守考量最壞的情境，亦即考慮風險值之 97.5 百分位數，男性攝食慣行栽培蔬菜所產生之 HQ 值 和標的致癌風險 TR 值分別為 0.82 和 1.31×10^{-5}，相較之下，女性之 HQ 及 TR 值則分別為 1.08 和 1.73×10^{-5}。由前述結果可知，在食用慣行種植的葉菜時，女性因硝酸鹽所造成之風險比男性高。若考量攝食有機蔬菜，則男性之 HQ 和 TR 之 97.5 百分位數值，分別為 1.77 和 2.83×10^{-5}，而女性的 HQ 和 TR 值，則分別為 2.23 和 3.56×10^{-5}。因此，當食用有機種植葉菜時，基於保守觀點，亦有一定的非致癌及致癌風險。此外，研究結果亦顯示在攝食葉菜類而暴露於硝酸鹽所產生的健康風險，女性比男性高。

本研究之結果，可作為臺灣制定蔬菜硝酸鹽含量標準之法規的參考依據之一。然而，在未來的研究中，建議對更多種類的葉菜類，乃至其他類型的蔬菜進行研究，另在討論不同類型蔬菜種類時，各類別的樣本數應盡量相同為佳。

關鍵詞：慣行栽培、葉菜類、有機栽培、硝酸鹽、風險

ABSTRACT

Vegetables can accumulate an enormous amount of nitrate during cultivation, especially leafy vegetables. Plant leaves require the presence of nitrogen in order to photosynthesize. As a result, a large amount of nitrate could build in the leaf and stem tissues, followed by the roots. Vegetables, on the other hand, are strongly suggested to be included in a human's diet due to their widely acknowledged health benefits. For this reason, vegetables are widely considered to be the most important source of nitrate intake. Today, dietary nitrate has been linked to an increased risk of fatal conditions such methemoglobinemia and gastric cancer. Thus, this research investigated the nitrate content of eight different vegetables that are widely grown and consumed in Taiwan. The goal of this study is to examine the nitrate concentrations of vegetables cultivated organically and conventionally, as well as to determine the impact of various farming practices on nitrate accumulation and concentration. This study calculated and compared the toxicological risk to people, mostly adult males and females, linked with the consumption of several vegetables containing varying levels of nitrate. The US Environmental Protection Agency's (US EPA) human health risk assessment paradigm was used to assess the non-carcinogenic and carcinogenic risk of Taiwan residents. Due to their large uncertainty and variability, the parameters were considered as distribution and the risk was calculated by Monte Carlo simulation using the Oracle Crystal Ball software. The results showed that the geometric mean and geometric standard of nitrate concentrations of vegetables grown organically were 996.02 mg/kg and 4.37, respectively, and 2497.97 mg/kg and 1.91 for conventional one (192 samples for conventional grown vegetables and 38 samples for organic grown vegetables). For non-carcinogenic risk, the mean value of the hazard quotient (*HQ*) of consuming conventional vegetables for male and female was 0.23 and 0.28, respectively. For the consumption of organic vegetables, the mean HQ for male and female was 0.30 and 0.36, respectively. This result indicated that the “average” non-carcinogenic risk for both groups can be considered “acceptable”. However, in the worst-case scenario, the 97.5%-tile values of *HQ* and target cancer risk (*TR*) of nitrate in males by consuming vegetables under conventional practice was respectively 0.82 and 1.31 x 10^{-5}, whereas the *HQ* and *TR* value for females was 1.08 and 1.73 x 10^{-5}, respectively. Therefore, it can be concluded that with the consumption of conventionally grown leafy vegetables, the female population is more prone to risk than the male population. As for the results on organic practice, the 97.5%-tile value of *HQ* and *TR* for males is 1.77 and 2.83 x 10^{-5}, respectively, while the female has a value of 2.23 and

3.56 x 10^{-5}, respectively. Thus, from a conservative viewpoint, consumption of organically cultivated leafy vegetables used in this study might put the target populations at risk. Furthermore, based on the results, it could be clearly seen that females are more likely to be exposed to risk than males due to the consumption of leafy vegetables.

The results of the present study can be applied while setting the local regulation standard for nitrate in Taiwan. However, experiments with more kinds of leafy vegetables and other types of vegetables are suggested in future research. It is as well a suggestion to use the same numbers of samples for future experiment.

Keywords: conventional farming, leafy vegetables, organic farming, nitrate, risk

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1 PREFACE

1.1 Introduction

Nitrogen is required for life on the planet. Approximately 78 percent of the Earth's atmosphere is nitrogen. Nitrogen plays a vital role in plants' structure, genetics, and metabolism and is one of the most abundant minerals. In crop plants, the element often produces a high yield response by stimulating quick vegetative development and giving the plant a healthy green appearance. However, plants cannot absorb Nitrogen in a gas form. There are different forms of nitrogen; still, plants benefit most from nitrogen in the form of nitrate. Bacteria convert nitrogen to nitrate, which is then taken up by plants during the natural nitrogen cycle. More than 90% of the nitrogen taken by plants is likely in the form of nitrate. For healthy plant growth, a sufficient quantity of nitrate is required. As a result, a lot of vegetables and forage crops accumulate a lot of nitrate.

Although nitrate is found in all aspects of our environment, such as air, water, soil, and the food we eat, the consumption of vegetables is generally regarded as the primary source of nitrate intake. However, despite the fact of being a large source of nitrate, increased consumption of vegetables is widely recommended due to its generally accepted health benefits. Known as a source of nutritious compounds, such as vitamins, minerals, and secondary metabolites, it contributes substantially to the human diet. Furthermore, vegetables are the most prominent dietary intake source, accounting for 70-80 percent of total nitrate intake by humans in an average diet.

Albeit nitrate appears to be non-toxic, when reduced and converted to nitrite, it can combine with amines and amides to generate carcinogenic nitrosamine compounds. An increasing number of studies reported that a high level of nitrate ingestion through vegetable consumption has been associated with the increasing risk of gastrointestinal cancers and methemoglobinemia (blue baby syndrome) that affects babies and children. High levels of nitrate and nitrite in food are now one of the most serious public health concerns, especially for vegetarian groups. Although nitrate and nitrite have potential health risks in previous studies, more recent studies have shown that nitrate and nitrite may also have potential health benefits.

For human health protection, according to JECFA (2016), the Joint FAO/WHO Expert Committee on Food Additives suggested that nitrate ions are not to be consumed in excess of 0.07 mg/ kg body weight per day. Furthermore, the European Union prescribed maximum nitrate limits for lettuce and spinach, which formed the basis of the subsequent regulation (No. 1822/2005) of the European Commission. Nitrate maximum level of concentration allowed for spinach ranges from 2000-3500 ppm; 3000-5000 ppm for lettuce; and rucola range 6000-7000 ppm. Therefore, examining and analyzing nitrate levels on different vegetables would be beneficial; furthermore, safe food needs to maintain nitrate levels in vegetables below legal limits.

The regulation of nitrate concentration in all kinds of vegetables has generated significant concern among researchers, especially farmers. Since crop yield is an important matter for production, farmers often use N fertilizers to have a good harvest. In soils, nitrogen is supplemented in the form of ammonium nitrogen which is quickly oxidized to nitrate (NO_3^-). The level of this compound in soil and water increases due to exposure to waste products from industrial processes, waste water, effluents, nitrogenous fertilizers, and herbicides. In consequence, Nitrogen supply and light exposure are therefore identified as the major determinants of vegetable nitrate content. Like so, researchers are encouraged to investigate further and to prove this theory.

In the case of Taiwan, official regulation on nitrates control in vegetables and managing the possible risk is lacking even though nitrate risks for public health have become an important issue and received increasing attention due to dietary exposures. However, the government has mandated limits on nitrites in smoked meats, yet no limits on freshly produced vegetables. Thus, the Homemakers United Foundation, an environmental and health issues Non-Government Organization (NGO), shared concern about nitrate levels and stated that the general public should be cautious of nitrates because of their potential to convert into a toxic substance.

1.2 Objectives

Due to the potential threat posed by excessive nitrate accumulation, determining the nitrate concentration of various vegetables, assessing the intake, and possible impacts on human health is highly crucial. Several studies pointed out that leafy vegetables could accumulate higher nitrate levels than any other kind of vegetables. Lettuce and spinach, such leafy vegetables, contain the highest concentrations of nitrate (Iammarino et al., 2014).

Thus, this study aims to achieve the following objectives:

1.) Examine the nitrate concentration on various leafy vegetables produced and highly consumed in Taiwan.
2.) Compare the level of nitrate in both farming practices (organic farming and conventional farming), and assess the relevance of these farming practices to the concentration of nitrate accumulated in the vegetables.
3.) Calculate the toxicological risk to Taiwan residents associated with the consumption of various vegetables containing various levels of nitrate, mainly for females and males.

The human health risk assessment paradigm established by USEPA will be used in the assessment. The possible results of the study could provide data to the government while setting the local regulation standard of nitrate in vegetables produced in Taiwan.

CHAPTER 2 LITERATURE REVIEW

2.1 Nitrate and Nitrite

2.1.1 Introduction to nitrate and nitrite

According to Gangolli (1994) and other authors, nitrate is not carcinogenic in and of itself, but once reduced to nitrite, it combines with amines and amides in the body to generate carcinogenic nitrosamine compounds, a process known as endogenous nitrosation. Nitrate's external sources include vegetables (Iammarino, 2014) and, to lesser extent water, although it is also generated endogenously to a limited level, according to Lundberg et al. (2004; 2008). On the other hand, nitrite intake is mostly due to the consumption of dietary additives found in cured meats. Nitrite is believed to positively affect the appearance, aroma, taste, safety, and raw meat quality. Ultimately, nitrite reduces lipid peroxidation (rancidity) and maintains meat flavor. "Curing," which means "to fix, restore, or cure," is the term for using nitrite in the preparation of meat products." As a result, it's a crucial component in the meat production process. On the contrary, vegetables exposed to nitrites that are derived from the natural conversion of endogenous nitrate seem to be unimportant and negligible (Council of Europe, 1993). Nitrate toxicity is known to be minimal, however, its metabolic conversion to nitrite has resulted in negative consequences (EC, 1997; EFSA, 2008). It is widely acknowledged that once taken, nitrates can convert to nitrites. When nitrites react with amino acids in the stomach, they form nitrosamines, a substance linked to cancer risk (Aires et al., 2013; Savino et al., 2006).

2.1.2 Evaluation, Legislation, and Acceptable Daily Intake

In 1961, the first international study analyzing the health concerns associated with nitrite and nitrate ingestion was done by the Food and Agriculture Organization/ World Health Organization (FAO/WHO) Joint Expert Committee on Food Additives (JECFA), reported by European Food Safety Authority (2008) and FAO/WHO (1962). The first legal limit on nitrate levels was imposed in Europe in 1975 in Council Directive 75/440/EEC to prevent methemoglobinemia in newborns caused by nitrate-contaminated water (Fan and Steinbers, 1996; Knobelock et al., 2000). As a result, the US Environmental Protection Agency (USEPA) set a

Maximum Contaminant Level (MCL) for nitrate at 44 mg/L (equal to 10 mg nitrate-nitrogen/L or 10 ppm). Vegetables' nitrate content became legally limited, and in 1997, the maximum amounts in vegetables were originally set by Commission Regulation in European Union in order to protect the health of the public (EC, 1997). However, the regulation has undergone multiple revisions. The European Union has defined several nitrate content limitations in spinach, rucola (arugula), and lettuce relying on the time of production. Fresh spinach collected from October 1 to March 31 has a maximum nitrate level of 3000 mg/kg, while spinach harvested from April 1 to September 31 has a maximum nitrate level of 2500 mg/kg. However, the maximum nitrate level for preserved, deep frozen, or frozen spinach is 2000 mg per kilogram. On one hand, the maximum nitrate levels specified for lettuce collected at various times of the year, similar to spinach but cultivated under cover and in the open air, range from 2500 to 4500 mg/kg. While the maximum levels for iceberg-type lettuce grown under cover and in the open air, respectively, are 2500 and 200 mg/kg. A limit nitrate level of 200 mg/kg was also established for processed cereal-based foods and baby foods for newborns and young children.

The Scientific Committee for Food reviewed the toxicological effects of nitrate and nitrite in 1990 and recommended an Acceptable Daily Intake (ADI) for nitrate of 0–3.7 mg/kg body weight (EC, 1992). In 2002, following a thorough assessment, The JECFA maintained an Acceptable Daily Intake (ADI) for nitrate of 0–3.7 mg/kg body weight and an ADI for nitrite of 0-0.07 mg/kg body weight (FAO/WHO, 2003a; FAO/WHO, 2000b). Following this, the USEPA then determined a Reference Dose (RfD) for nitrate and nitrite of 7 mg/ kg body weight per day and 0.33 mg/ kg body weight per day, respectively (Mensinga et al., 2003). The World Health Organization (2008) advises a daily diet of 400 grams of fruits and vegetables per person, with vegetables being high in several key elements that protect against chronic diseases.

2.1.3 Accumulation and concentration of nitrate in vegetables

Albeit nitrate is known to be a crucial component of plant material, it still can accumulate in plant tissues. Because nitrate is exclusively carried by xylem, the majority of the nitrate is stored in the mesophyll cells of the leaves while the level of nitrate in fruits and seeds is low (Gorenjak and Cencic, 2013). According to EFSA (2008), water and nutrients are absorbed by roots to leaves via the xylem, while the phloem carried photosynthetic products from the leaves to the plants' growth point. These impacts nitrate distribution between leaves and storage structures like seeds and tubers. This means that leafy vegetables like cabbage, spinach, and lettuce have high nitrate levels. Storage organza, on the other hand, has low concentrations, such as bean plants, pods of peas, carrots, onions, potato tubers, and leeks. Santamaria (1999) stated that concentrations of nitrates differ in plant's various parts. Santamaria (2001) experimented then listed vegetable parts that contains the lowest to the highest level of nitrate as follows: seed< fruit < bulb < tuber < inflorescence < root < stem < leaf < petiole. Furthermore, Santamaria (2006) classified the vegetable families that accumulate high amounts of nitrate as Asteraceae (artichokes, lettuce) and Apiaceae (carrots, celery, coriander, parsley), Brassicaceae (arugula, cabbage, rape plant, radish, mustard), and Chenopodiaceae (beets, spinach, swiss chard); Amaranthaceae species, also been included with high nitrate contents. The experimental study performed by Aires et al (2013) verified this pattern.

Green leafy vegetables have been consistently reported to possess the greatest nitrate level. When compared to other vegetables, the levels of nitrates and nitrites in dark green leafy vegetables were found as significantly higher. (Hord et al., 2009; Ranasinghe and Marapana, 2008). Which was validated by Gonzales et al. (2010), who found out that nitrate levels in leafy vegetables (particularly Swiss chard species) were substantially higher than in inflorescence and fruit products. It's also been claimed that spinach has high nitrate concentrations, around 1000 ppm on average, and up to 3000 ppm in rare instances (Zhong et al., 2002). A study conducted by Abo Bakr et al. (1986) used sixteen fresh vegetables from several categories, including leafy vegetables, pulses, root vegetables, and others, to determine the amounts of nitrate and nitrite in several regularly eaten vegetables in Egypt. In comparison to pulses and root vegetables, leafy vegetables have higher quantities of nitrates (the highest value was found in spinach, at 5830 ppm). Many studies, especially in Europe and the USA, have demonstrated that leafy vegetables are generally rich in nitrate (SCF, 1997; Triantafyllidis et al., 2008). Other vegetables, such as oilseeds, cereals, and nuts, include nitrate in addition to leafy vegetables that may contain substantial levels (Brkić et al., 2017).

2.1.4 Factors influencing the accumulation and concentration

Nitrate accumulation in crops is affected and influenced by a variety of factors including physiological, environmental, and nutrition factors. According to Aires et al. (2003) and Tamme et al. (2006), nitrate accumulation is influenced by weather conditions, fertilizer application and cultivation, soil qualities and properties, harvesting time, storage conditions, and size of vegetables, in addition to the agriculture system and practices, in which concentration is also dependent. Growth density, air temperature, moisture, duration of the growth period, and edible plant portion also affect the concentration of nitrate in vegetables (Hsu et al., 2009; Reinek et al., 2009, Tamme et al., 2006). Nitrate levels differ between species, varieties, and even genotypes with different chromosome set levels.

Cantliffe (1973) stated that Nitrogen fertilizer and light intensity have been regarded as significant variables influencing nitrate content in vegetables among the parameters evaluated that affect NO_3 acquisition and storage in vegetable tissues. Several research and experiments concluded that vegetables produced and grown in warm areas like greenhouses have higher nitrate content compared to those grown outdoors (Thomson et al., 2007). Light intensity and soil nitrate concentration are particularly significant variables in generating nitrate levels in spinach or other leafy green plants (Santamaria et al., 1997; Santamaria et al., 2001). According to Gruszecka-Kosowska (2017), unfavorable light settings significantly affect the ability of leafy vegetables with a shorter growing season to acquire nitrates in the leaves. Liu et al. (2014) also concluded that nitrogen fertilizers' application influences the concentration of nitrate in lettuce’s edible sections. Vegetables, particularly green leafy vegetables like lettuce, spinach, and rocket (rucola) (Hord et al., 2009; Ranansinghe et al., 2008; Reinek et al., 2009), and cured meat products (Santamaria, 1997) absorb more than 80-95 percent of nitrates. Plant foods include 1-2 mg of nitrite per kilogram of fresh vegetable; potatoes, on other hand, can contain up to 60 mg of NO_2^{-1} per kilogram of fresh vegetable weight (Walker, 1996). The content of nitrate in fresh vegetables is usually low. Because the inner reductase is inactivated in cold circumstances, nitrite buildup in plants is reduced (Chan, 2011). However, Santamaria (1997) reported after several days at room temperature, greater nitrite levels in contaminated food and damaged vegetable fibers were detected. Other authors also stated that when a vegetable is refrigerated for about 12 hours or more might result in an even higher level of nitrite due to the release of powdered reductase from internal nitrate. Even so, some methods were introduced to effectively reduced the amount and prevent the build-up of nitrate and nitrite in certain vegetables. Such methods are removal of stem and midrib commonly in lettuce and spinach; peeling of potatoes, beetroot, and other root and tuber crops; and cooking and blanching of some vegetables.

Furthermore, when the plant grows so slowly that it does not metabolize into proteins will cause nitrate to accumulate in the soil when its concentration is excessive. Even in soils with moderate nitrate levels, slow growth can result in nitrate accumulation, in addition to too much nitrate in the soil.

2.1.5 Exposure to nitrate

There are numerous chemical, agent, and pollutant sources in our surroundings that may pose a threat. They could be carried to the target through food, water, air, or dermal contact. Thus to assess the risk it is crucial to understand how humans are exposed to those substances. Route of exposure can be through inhalation, dermal contact or absorption, and ingestion. It is by ingestion in the case of nitrate in vegetables.

Low concentrations of nitrate are generally considered harmless. Nitrite, in contrast, is a reactive molecule that nitrosates other molecules such as proteins, amines, and amides in the stomach's acidic environment. Nitrite can be found in the environment on rare occasions, but the majority of humans are exposed to it through the ingestion of nitrate, which is chemically transformed to nitrite by commensal bacteria found in saliva (Hyde et al., 2014). Among the dangers of nitrate and nitrite is the concern about preformed N-nitrosamines and N-nitrosamines created in the stomach after swallowing meals high in nitrate and nitrite. By reacting with nitrite, some low-molecular-weight amines can become their carcinogenic N-nitroso counterparts, resulting in this concern.

A complicated entero-salivary route converts ingested nitrate to nitrite. In summary, nitrates are absorbed by the digestive tract and enter the circulation, where the salivary gland takes them up via blood circulation. When nitrate reaches the saliva, commensal bacteria actively convert it to nitrite (Lundberg and Weitzberg, 2013). A human's salivary nitrate is secreted at a rate of about 25%, and about 20% of salivary nitrate is converted to nitrite by microbes on the tongue (EFSA, 2008; FAO/WHO, 2003a). According to Duncan et al., (1995) nitrate reduction occurs most effectively at the tongue base, where a stable, nitrate-reducing microbiota exists.

2.1.6 Effects of nitrate on human health

Many studies are still in discourse about the carcinogenicity of nitrate and nitrite in food, particularly in vegetables. The link between nitrite and cancer appears to be ambiguous. High nitrate intake, on the other hand, has been tied to a surge in cancer cases of the urinary bladder, esophagus, nasopharynx, prostate, colon, and gastrointestinal regions, and oral cancers (Michaud et al., 2004; Turkdogan et al., 2003). The capacity of nitrite to react with hemoglobin (oxyHgb) to produce methemoglobin (metHgb) is perhaps its most well-known impact (Gorenjack and Cencic, 2013). Methemoglobinemia is a condition in which hemoglobin's reduced iron (Fe+2) is oxidized to Fe3+, resulting in methemoglobin production. The oxygen transport to tissue is harmed as a result of this. Due to lower acidity, which favors the growth of nitrate reduction bacteria, and the presence of fetal hemoglobin, which is easily oxidized by nitrate, the most vulnerable to this illness are babies 3 months below and also young children (Chan, 2011; Ranasinghe & Marapana, 2008; Santamaria, 2006). Gorenjak & Cencic, (2013) stated that infections of the gastrointestinal tract in infants may result in an increase in nitrate to nitrite conversion.

A lot of research has been done on the relationship between dietary nitrates and nitrites and cancer, particularly gastric and other gastrointestinal malignancies (Mirvish, 1995). In the United States, the National Research Council found a link between nitrate consumption and gastrointestinal and esophageal cancer (Mohammadi and Ziarati, 2016). A study by Hsu et al., (2009) found that stomach cancer rates varied dramatically across nations; for instance, in Japan, the rate was seven times higher than in the United States, United Kingdom, and Germany. Joosens et al. (1996) also reported that South Korea, China, and Columbia have the highest rates of stomach cancer death among men. The increased incidence of stomach cancer in the Far East may be due to the consumption of nitrate-rich diets. In Korea, for example, kimchi is a popular side dish for the majority of people. Kimchi is a Korean relish made from cabbage, radish, cucumber, mustard leaf, green onion leaves, and other ingredients. Koreans consume this type of food on a daily basis, along with a variety of veggies. In South Korea, regular use of salted, pickled cabbage, and salted seafood sauce was associated with a greater risk of stomach cancer. Duncan et al. (1997) stated that some food preparation, for instance, broiling meat and eating foods heavy in salt as in many traditional Japanese dishes, may lead to a nitrate-rich diet. Hsu et al. (2009) also discovered total N-nitroso compound precursors in substantial proportions in salted seafood sauce, despite the fact that cabbages are already known to be high in nitrate. However, the study conducted in Korea by Kim et al. (2007), where dietary nitrate intake (390-742 mg/day) is significantly higher than in European countries (52-156 mg/day) and China (422.8 mg/day), concluded that dietary nitrate consumption is not associated with cancer.

Aside from these disadvantages, Hord et al. (2009) reported that dietary consumption of nitrate and nitrite from vegetables and fruits may contribute to blood pressure decrease through Dietary Approaches to Stop Hypertension. Pragani et al. (2017) added that nitrate and nitrite were demonstrated to aid in the prevention of ischemic heart disease by increasing epicardial blood flow, decreasing vascular resistance, blunting coronary steal, and lowering preload. Carlström et al. (2011) found that nitrate in meals can lower oxidative stress and protect the heart and kidneys. A study conducted by Song et al. (2015) concluded that dietary nitrate may reduce the risk of gastric cancer, possibly because vegetables are the major source of dietary nitrate and also of health benefits, such as vitamin C, fiber, and other reducing agents. According to Bottex et al. (2008), nitrosamines can be reduced by half when nitrates are ingested.

The beneficial properties of nitrate, nitrite, and metabolites are present in most nitrate-rich vegetables, such as lettuce, spinach, and beetroot. According to Bazzano et al. (2008), consumption of green vegetables is linked to a lower risk of diabetes in women, whereas Larsen et al. (2007) stated that nitrate-rich vegetables have been shown to minimize oxygen requirements throughout the activity. Hord et al. (2009) also added that nitrate and nitrite should be regarded as nutrients. Various epidemiological researches deny the link between nitrite and stomach cancer (Jakszyn & González, 2006); but nevertheless, nitrate has both a favorable and unfavorable impact. On the intake, these functions predominate (Gorenjak and Cencic, 2013). Apparently, excessive intake represents a risk. Too much of anything is never a good thing.

2.2 Comparative studies on farming practices

The Scientific Commission on Food recommended that since nitrate can become nitrite and nitrosamine when digested in food and water, continuous efforts must be made to reduce exposure to nitrate. SCF (1995;1997) has advocated for the use of GAP or Good Agricultural

Practices to keep nitrate levels as minimal as possible. According to Santamaria (2006), increased application of artificial nitrogen fertilizers and animal waste such as pig manure and cow dung in intensive agriculture may result in higher content of nitrate in vegetables and drinking water than those in the previous times, implying that rational application of nitrogen fertilizer should be considered in farming to avoid the build-up of nitrate in soil and vegetables. Yet, producers and farmers are constantly struggling to maintain nitrate concentrations below regulatory levels (Aires et al., 2013).

Organic farming is widely regarded as one of the most efficient methods to reduce food-related health concerns (Gruszecka-Kosowska & Baran; 2017; Murawska et al., 2015). However, organic farming and conventional farming practices need Nitrogen(N) as numerous kinds of field crops require nitrogen, which is acquired in the nitrate form by crop plants. Thus, to ensure the yield of marketable production, farmers tend to widely apply nitrate fertilizers to crops (Agostini et al., 2010). Thus, over-fertilization with nitrogen is the primary cause of the enormous nitrate accumulation in crops (Bian et al., 2020). It was also stated on Human Health Fact Sheet (2005) that high usage of nitrogen-containing fertilizers, as well as intensified livestock and poultry husbandry, are only a few human activities that contributed to the increase of nitrate concentrations in the environment. Even so, multiple studies have shown that many individuals believe organic foods are healthier and are produced in a more acceptable manner than conventionally farmed goods. Even in Europe, organic products are becoming more popular as a result of the lack of chemical toxins in this method of production (Rembialkowska, 2007).

Produced organically means food that has not been exposed to excessive chemical inputs such as inorganic fertilizers, pesticides, and herbicides, as well as food that has not been genetically modified organisms (GMO) (Aires et al., 2013; EU SCF, 1995). However, according to Pussimier et al. (2006), humans must be reminded that Organic farming does not adhere to the restrictions of the production system that prohibits (or substantially minimizes) the use of certain chemicals. Though this production intends to respect more accurately a balance between man, and the environment, it still has to meet the global quantity and quality requirements. On the other hand, conventional farming includes synthetic fertilizers, pesticides, herbicides, and other continual inputs that are harmful to humans and the environment. In addition, Pussimier et al. (2016) noted that "conventional" refers to a wide range of production systems with a variety of technical characteristics (classical production requiring intensive inputs as well as alternative production systems utilizing Integrated Pest Management (IPM), production of goods with a label or with marketing claims, and production according to specifications established by the distribution sector).

As an alternative to traditional agriculture, organic production focuses on the incorporation of organic material into the soil, with animal dung being the most common fertilizer (EU Scientific Committee for Food, 1995). Manure from animals is an excellent source of macro and micronutrients, notably Nitrogen. Organic products should, in concept, have lesser nitrate levels compared to their conventional counterparts (Aires et al., 2013; Gonzalez et al., 2010). As a result, organic farming is continuously evolving, necessitating a transition to a greater scale than the family farm.

However, significant differences between organic and conventional foods regarding nutritional properties and health impacts are still in dispute in different studies. Comparative studies have shown that organically grown veggies had lower nitrate levels than conventionally grown vegetables. Results of the study conducted by Guadangnin et al., (2005) also reported that hydroponic lettuce had more nitrate concentration than lettuce grown in conventional systems, which had higher nitrate content than lettuce grown in organic systems. On the other hand, several research found the opposite. Many studies have found substantially greater levels of nitrates in organically grown foods than in conventionally grown foods (De Martin & Restani, 2003; Gorenjak & Cencic, 2013). Such as the study conducted by Gruszecka-Kosowska & Baran (2017) indicates that nitrate concentrations in leafy vegetables such as celery, spinach, kale, broccoli, lettuce, etc. were greater in organically grown compared to conventional farming. Yet, several studies have found that the level of nitrates in organic and conventionally grown vegetables is similar. For instance, the findings of the study conducted by Aires et al. (2013) showed that baby leaf salad grown in both farming systems in Northern Portugal has the least level of nitrate and nitrite. The same goes with the results of a recent National US investigation of raw vegetables classified as conventional and organic at retail, which has found no difference in nitrate and nitrite concentrations between conventionally and organically produced lettuce, cabbage, broccoli, and celery (Nunez et al (2015). Spinach, on the other hand, is excluded from the group since its nitrate content in organically produced is 1318 ppm, which is lower than its conventional counterpart (2797 ppm).

Although the difference between organically and conventionally grown plants is vague, it does exist. Despite the growing popularity of organic farming, there isn't enough information or evidence to say conclusively that the risk of nitrate or nitrite accumulation in organic farming is the same as the risk in conventional farming. This is due to a number of important factors that must be considered when conducting comparison research and, as a result, when interpreting the results. Whether crops are farmed organically or conventionally, numerous factors influence their nutrient density. Some factors have an equal impact on both production systems, whereas others have a greater impact on one than the other. Domagala-Swiatkiewicz (2013) also mentioned that in order to conduct a legitimate comparison between organic and conventional agricultural food items, the plant must be the same cultivar and must be grown in close proximity to one another, in similar soils, and under similar climatic circumstances.

2.3 Human Health Risk Assessment

2.3.1 Risk

Risk is defined as the probability that an individual, population, or ecosystems will be harmed or experience adverse effects after being exposed to toxic substances or to hazardous conditions. An adverse effect could be defined as death or disease in the case of human risk analysis. It usually is an unfavorable and disastrous consequence of something. Exposure is characterized as the contact of an organism or human in the case of health risk assessment) with a chemical or physical agent. While hazard refers to the source of risk, such as toxic chemicals or toxic substances. For example, people use air fresheners daily at home. Though it may emit pleasant aromas and essences, air fresheners are in fact, made from ethylene-based glycol ether and terpenes. Though ethers are regarded as toxic themselves by the EPA, terpenes are non-toxic, however, when reacted with the ozone in the air, could produce a poisonous combination. Thus, air freshener is a toxic substance that could be a hazard to human health. Even so, if the air freshener is kept closed, the risk of this hazard is zero, since human is not exposed to the chemicals. On the other hand, when the container of air freshener is opened, the risk linked to it is greater than zero. Risk is upon being exposed to that toxic substance. To make it short, the risk is determined by the toxicity of the substance and the exposure to it.

The United States Environment Protection Agency stated that risk is dependent on three factors: the number of stressors (arsenic, mercury, lead, etc.) in the environment; the exposure of humans to the contaminated medium; and the effects on human health and ecological receptors due to toxicity. Considering that humans are constantly exposed to hazards through poisonous components or substances, it is critical to assess the risk based on the negative consequences they have. As a result, ongoing research and study are encouraged to uncover some other hazardous elements that could put humans at risk. Risk assessment, management, and analysis are all concepts that are associated with risk.

2.3.2 Risk Assessment, Risk Management, and Risk Analysis

Other authors define risk assessment as a strategy for evaluating and determining the potential consequences of a substance on human health. It's a method for determining and describing how harmful a chemical or hazard is. Many risk assessments are carried out in order to determine a safe level of exposure or to assess the likelihood of a group of persons being harmed as a result of exposure.

To build case-specific responses to environmental pollution, the risk assessment method will often rely on factual information. As for EPA, risk assessments are typically split into one of two areas: Human Health risk assessment or Ecological assessment.

According to the Victorian Government's Department of Health, Human health risk assessment is a way of evaluating the potential impact on human health as exposure to hazards. It is also worth noting that every human health risk assessment is distinct and dependent on the scenario and demographic being assessed. Individuals or specific groups such as infants, children, teens, the elderly, or individuals with specific health issues such as chronic bronchitis, for example, maybe the target demographic. Risk assessors should acquire factual information regarding who, where, and what is at risk when conducting Human Health Risk Assessment. This could be an individual, a subgroup, or the entire population. It's also crucial to understand the concern about the environmental hazards, which could be radiation, toxins, or a nutritional component, as well as where these environmental hazards originate. Then exposure pathways are identified, which may include air, soil, waste, groundwater, food, or pharmaceutical goods. Identification of routes is just as important as pathways, which could include ingestion, inhalation, or skin contact (absorption), depending on human behaviors that lead to exposure to a toxic substance. Furthermore, the chemical's health implications should be determined. And also the duration of its harmful effect, which could be acute stage immediately, or might sub-chronic for weeks, or chronic for the rest of one's life. When evaluating risks, risk assessors analyze a variety of factors, but there are just four basic processes to any risk assessment, including Hazard Identification, Dose-Response Assessment, Exposure Assessment, and Risk characterization. The later section of this chapter will go through each of these steps.

An ecological risk assessment, on the other hand, is the process of determining the likelihood of negative ecological effects when exposed to one or more environmental stressors such as pollutants, infections, and invasive species. There are three stages involved in an ecological risk assessment. However, before proceeding to these stages, **Planning** should be the primary step. Planning involves designing and framing the entire strategy with interaction among risk managers, risk assessors, and perhaps other interested individuals or participants. Team members will determine risk management goals and alternatives, as well as natural assets of interest, the scope of the assessment, and the duties of every member of the team. After the planning, assessors could proceed to the first phase, which is **problem formulation**. In this phase, risk assessors collect data to identify whether plants and animals are in danger and require protection. They stipulate the scope of work in terms of the time or space, as well as the environmental factors regarded, the parameters, metrics, approaches, and kinds of data which will be used to evaluate the risks to those endpoints based on the results of Planning. Then, a plan for analysis follows the articulation of the problem. **Analysis** is the second phase. Exposure and effects assessments are two aspects of the analytical process. Risk assessors evaluate during exposure assessment, whether plants and animals are susceptible to every environmental stressor and to what extent they are responsive. During the assessment of the impacts, the risk assessors evaluate the pieces of literature on the relation of exposure level and potentially detrimental effects on both plants and animals. They may also look at evidence of existing negative environmental effects. **Risk Characterization** is the third and the last phase. Risk estimation and risk description are the two main components of risk characterization. In risk estimation, the projected or determining levels of exposure for every stressor, and also plant and animal population, group, or ecology of concern are compared to the data on possible impacts for those certain groups. While risk description provides crucial data about the outcomes of risk analysis. This includes determining whether detrimental effects are expected on the plants and animals of interest; making appropriate qualitative comparisons; determining how might assumptions such as data gaps and natural variation influence the evaluation. Risk assessment's major purpose is to provide risk managers with all of the data they need to make the best decision possible in a potentially harmful situation. Finally, the risk assessment creates a framework for collecting the risk data needed to make decisions.

In the process, risk assessment outputs are merged with factors such as economic, social, and legal issues to make decisions needed that are feasible in implementing various risk reduction strategies and measures. Simply put, risk management is the application of risk assessment outputs in an effective manner. Risk management, according to Environmental Protection Agency, is a process that examines possibilities for safeguarding human and environmental health. Evaluation of alternatives; selecting the most effective response in diminishing risk; developing a plan to implement this action, executing the plan, and monitoring the execution process to ensure that the desired outcome is achieved and maintained as planned are some of the other functions of risk management. Risk managers use the results of risk assessments to communicate to all parties concerned, as well as the broader public. Risk assessors, policymakers, and other employees discuss risk with one another in a process known as Risk Communication. Risk assessors and risk managers may communicate, as well as risk assessors, managers, and citizens. Risk assessment, risk management, and risk communication are three interconnected areas needed to address the risk imposed by the environmental hazard to human health.

Lastly, a broad term that involves risk assessment, risk management, risk communication, and comparative risk analysis, is called Risk Analysis. Risks compared to one another, including individual risks or groups of risks is known as Comparative Risk Analysis.

In general, risk assessment seeks to answer three fundamental questions: what might possibly go wrong (scenarios)? What are the chances that will happen (probability)? And, if this does happen, what are the expected consequences (adverse effects)? Answers to these questions could be generated with a complete risk analysis linked to a certain scenario or activity. As such, risk analysis plays a vital role in addressing the risk as it considers the processes performed in risk assessment, risk management, and risk communication before making a decision and setting a policy.

2.3.3 Process for Human Health Risk Assessment

Basically, there are four steps in executing a human health risk assessment generated by the United States Environmental Protection Agency. These are hazard identification, dose-response assessment, exposure assessment, and risk characterization, in order.

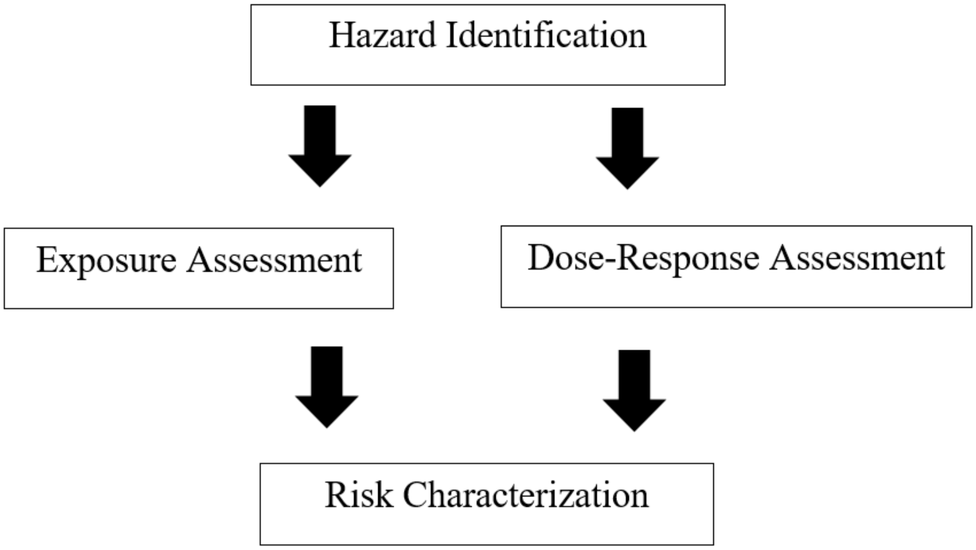

Figure 1. Human Health Risk Assessment paradigm proposed by USEPA

- Hazard Identification - a strategy used for determining the population affected, how they are being affected, and whether exposure to that specific agent has the potential to pose detrimental effects on individuals (e.g., birth defects, cancer) or ecosystems in constrained conditions, or whether the unfavorable health consequence would most likely occur in humans. Analyzing the nature and strength of the evidence of causality is an important aspect of hazard identification. It should provide a response to the question "What the chemical does?"

- Dose-Response assessment - explains the relationship between dose and incidence in humans. Toxicity metrics such as reference dosage and slope factor are calculated from this quantitative dose-response relationship which is useful in predicting the occurrence of adverse effects when humans are exposed to toxicity. The reference dose (RfD) estimates the daily exposure level of the human population. RfD is affected by the mode of exposure (oral or inhalation), the critical effect (developmental), and the duration of exposure (chronic, sub-chronic, or single event).

- Exposure Assessment - examines the frequency, duration, and levels of exposure to the chemical. This step determines the population affected and how they are exposed to hazards. In this step, the exposure point and exposure route are determined. Exposure point shows when and where humans are exposed to certain agents. On one hand, the exposure route explains how humans are being exposed, whether through ingestion, inhalation, dermal contact, or absorption. Estimation and calculation of exposure concentrations and intakes are some other factors performed in this stage of assessment.

- Risk Characterization - determines the amount of pollutants people are exposed to during a specific time period and the number of people exposed. In this stage, the level of risk will be identified. Thus, Hazard Index (HI) is used in this step to characterize potential noncarcinogenic effects. HI is generated by dividing the intake over RfD. And when the result of the hazard index is 1 or greater it is then considered harmful. The essential step in risk characterization is the identification of the key parameters influencing risk. One method effectively used in this regard is sensitivity analysis. Sensitivity analysis displays the values of parameters that influence risk and cause a change in risk. The results are summarized and compared. These comparisons may be the basis for the conclusions of the study, or they may indicate that more data should be needed to resolve the problem.

2.4 Monte Carlo simulation

Monte Carlo provides an excellent method for evaluating estimators and goodness-of-fit statistics under a variety of conditions, including sample size, nonnormality, dichotomous or ordinal variables, model complexity, and model misspecification (Paxton et al., 2001). According to Qui et al. (2021), Monte Carlo simulation works well at modeling the probability of the different outcomes that previously were difficult to predict because of the influence of random variables. This method was applied in the human health risk assessment, to better understand the impact of the risk, and to reduce the randomness and uncertainty in prediction. Monte Carlo simulation also provides sensitivity analysis to identify the most influential variables. 10,000 iterations can be used to run a model to obtain the probability distributions.

CHAPTER 3 MATERIALS AND METHOD

3.1 Sample collection

In the experiment, eight different types of green vegetables were harvested and employed. A total of 230 samples were collected, with 192 being produced conventionally and the remaining 38 being farmed organically. Spinach, amaranth, napa cabbage, sweet potato leaves, pak choy, a-choy, bok choy, and rape plants are all leafy vegetables. To guarantee that the veggies used in the experiment were 100% organic, they were purchased from an organically qualified market that has its official CAS (Certified Agricultural Standards) marking for organic Taiwanese products and displays its certification recognized by the Council of Agriculture. Conventional vegetables were merely purchased in the market.

3.2 Nitrate concentration analysis

The analysis was performed by Professor Cheng-Wei Liu, Chair of the Department of Post Modern Agriculture at MingDao University. Vegetables are obtained, then subjected to experiments, so the vegetables used are raw and fresh. Ion chromatography was used to measure nitrate concentrations. The results of the analysis on nitrate concentration per sample are shown in chapter 5 of this study.

3.3 Risk Assessment

The assessment was performed based on the paradigm by the United States Environmental Protection Agency. This includes hazard identification, dose-response assessment, exposure assessment, and risk characterization.

3.3.1 Hazard Identification

Nitrate intake is linked to humans' consumption of vegetables, however, this study mainly focuses on leafy vegetables. Taiwan citizens are chosen as the target population in this area, primarily adult males and females. These people ranged in age from 19 to 65. Because nitrate exposure is caused through vegetable consumption, the exposure pathway is found to be ingestion. To assess the potential effects of nitrate, the ingestion rates of vegetables for males and females are determined, which are 303.76 g/day and 309.12 g/day, respectively. This data can be found in Taiwan's National Feeding Database. This parameter, along with the generated vegetable concentrations and the population's average body weight, will be utilized to compute the daily nitrate intake for both groups subsequently. The results of daily intake are important in determining whether nitrate is carcinogenic or not, even though the Integrated Risk Information System concluded that nitrate is not carcinogenic to humans.

3.3.2 Dose-response Assessment

In this study, the dose-response relationship or exposure-response relationship is not calculated. Rather, the existing Reference dose (RfD) for ingestion of nitrate set by IRIS with the value of 1.6 mg/kg/day was applied. For slope factor (SF), the suggested value of some authors was used, rendering a value of $1x10^{-5}$(kg*day)/mg. Both RfD and SF are used in characterizing the risk posed by nitrate intake.

3.3.3 Exposure Assessment

For exposure assessment, Daily Intake was calculated using several parameters. The average body weight (BW) of adult males and females in Taiwan, nitrate concentrations in organic and conventional vegetables (Cveg), and ingestion rates (IR) of vegetables are among the parameters utilized. The conversion factor is also taken into account in order to attain the DI unit of mg/kg/day. In the table below, each parameter's value and associated unit are listed. The equation used in calculating the DI is:

$$DI = (Cveg \text{ x } IR \text{ x } CF)/BW$$

Table 1. Parameters used in exposure assessment

Parameters	Symbol	Input Value	Unit	Source
Ingestion Rate	IR	Male- LN (9.81, 1.22) * Female- LN(9.81, 1.22)	g/day	National Feeding Database
Body Weight	BW	Male- N (72.30, 18.16) ** Female- N(58.40, 17.16)	kg	潘文涵 (2016)
Vegetable Concentration	Cveg	Organic- LN (996.02, 4.37) Conventional-LN(2497.97, 1.91)	mg/kg	this study
Conversion Factor	CF	10^{-3}	kg/g	

*Lognormal Distribution (mean, standard deviation); **Normal Distribution (mean, standard deviation)

The average body weight of male and female differs significantly. Corresponding values for male and female body weight are 72.30 kg and 58.49 kg, respectively. Monte Carlo simulation analysis was performed to modify the underlying parameters. In calculating the Cveg and IR value, a Lognormal distribution was applied to avoid non-positive values as the data are too large. The geometric mean and geometric standard deviation of the said parameters were used in the calculation. However, a Normal distribution was used in calculating body weight. 10,000 trials were performed to obtain a 97.5 percentile. Values for 2%, 25%, 50%, and 75% were also generated to observe the underlying results.

3.3.4 Risk Characterization

Since nitrate was considered non-carcinogenic by the Integrated Risk Information System, the Hazard Quotient (HQ) should be calculated using the Reference Dose implemented by the IRIS database, which is 1.6 mg/kg/day. The Hazard Quotient defines non-carcinogenic risk on human health posed by exposure to a corresponding toxic agent or element.

To calculate HQ, the equation used is:

$$HQ = DI / RfD$$

DI is the value obtained during exposure assessment. USEPA set an acceptable limit of non-carcinogenic, which is 1. If the HQ is <1, then non-carcinogenic health effects are not assumed to happen. A value of HQ greater than 1 indicates that there may be adverse health effects associated with the exposure, though there is no statistical likelihood that non-carcinogenic health effects will occur. If the value of HQ is greater than 1, it is therefore risky.

Target risk meanwhile is calculated to assess the potential risk associated with exposure to carcinogenic agents. The equation used in calculating TR is

$$TR = DI \times SF$$

where the oral slope factor is utilized. In this study, an existing slope factor of 10^{-5} is used (Duvva et al., 2021). The permissible limit of cancerous risk set by USEPA is $1x10^{-6}$ (kg*day)/mg for ingestion or oral intake. TR with a value of greater than $1x10^{-6}$ is considered risky and carcinogenic. The DI is required in order to calculate the HQ and TR, which are then analyzed using Monte Carlo simulations. To calculate the average-case and worst-case values, 10,000 trials are conducted.

Table 2. Parameters used in risk characterization

Parameters	Symbol	Input Value	Unit	Source
Reference Dose	RfD	1.6	(mg/kg)/day	IRIS database
Slope Factor	SF	10^{-5}	(mg/kg)/day	Duvva et al. (2021)

3.4 Statistical Analysis

This study used Monte Carlo Simulation for statistical analysis. Using Monte Carlo simulation, each provided value is calculated according to a probability distribution, which was determined using the goodness-to-fit test. A lognormal probability distribution was fitted to the parameters of vegetable concentrations and ingestion rate. This is to provide a better fit to some variables than what the normal distribution does and to avoid negative data. Instead of using the mean and standard deviation, geometric mean and geometric standard deviation were used instead. Meanwhile, body weight was fit using the normal distribution. This is because the bodyweight presented has both the mean and standard deviation. In the following step, Daily Intake was computed based on the generated values of Cveg, IR, and BW. DI was then forecast to predict and calculate possible future values. The same goes with Hazard Quotient and Target Risk after calculating the results. 10,000 iterations were then performed to obtain a 97.5 percentile, which is needed to assume the worst-case scenario.

Monte Carlo simulation was carried out through Oracle Crystal Ball software (version 11.1).

CHAPTER 4 RESULTS AND DISCUSSION

4.1 Nitrate concentration in vegetables

In this study, analysis shows that nitrate concentration was lower in organically grown leafy vegetables than in conventionally grown leafy vegetables. The table below shows that a 60% lower nitrate concentration was observed in organic spinach; almost 80% for sweet potato leaves; about 33% for rape vegetables; nearly 30% lower for Pak Choy; practically 40% for A-Choy; and about 65% lower for Bok choy. However, two kinds of leafy vegetables grown organically were observed with higher nitrate content than their conventional counterpart. Organic Amaranth has 29% more nitrates than conventional ones. At the same time, organic Napa cabbage was about two times more nitrate content than conventional Napa cabbage. Of the vegetables surveyed, the highest nitrate concentration was obtained in hydroponically (non-organic) grown Pak Choy (6829 mg/kg), 33% higher than the organic Pak Choy and followed by Rape plant with a nitrate content of 5241mg/kg and 3520 mg/kg for conventional practice and organic practice, respectively. Organic Sweet potato leaves have the lowest level of nitrate, with a content of 326.

Table 3. Nitrate content in organic leafy vegetable

Samples	**Number of samples**	**Range**	**Mean**
Spinach	3	423-1500	1121
Amaranth	9	480-6600	3272
Napa Cabbage	9	510-6997	3048
Sweet potato leaves	9	175-527	326
Rape	2	2804-4235	3520
Pak Choy	3	4035- 5384	4892
A-choy	2	862- 922	892
Bok choy	1	1185*	1185

*one sample available

Table 4. Nitrate content in conventionally grown leafy vegetables

Samples	**Number of samples**	**Range**	**Mean**
Spinach	29	435-2947	2864
Amaranth	30	76- 5715	2528
Napa Cabbage	31	77- 777	1559
Sweet potato leaves	28	16- 5097	1571
Rape	4	3850- 6750	5241
Pak Choy	6	4852- 9193	6829
A-choy	38	45- 4497	1508
Bok choy	26	540- 5855	3250

Based also on the results, accumulated nitrate content on spinach contradicts to most studies' typical result where spinach usually got the highest amount of nitrate. This study's analysis of nitrate content in both organic and conventional spinach does not exceed the limits (2000-3000 mg/kg) established by European Union. In addition, it concedes with the result of the National US Survey as stated in the introduction, where nitrate level in organic spinach (1318 ppm) was lower than its conventional counterpart (2792 ppm).

There is a clear finding in this study confirming a lower nitrate content in leafy vegetables from organic cultivation and higher in conventionally cultivated vegetables. The same result was found in some other studies. The comparison of the nitrate accumulation in organically and conventionally cultivated vegetables in the research conducted by Triantafyllidis et al. (2008) indicated statistically significant differences in lettuce and spinach (accumulation in conventional samples higher than in organic samples). The same goes with Basker (1992) who performed a comparative study in Austria using 17 vegetables and found lower nitrate contents (40%-86%) in organic vegetables. Pussemier et al. (2006) also conducted a study and reported that organic and conventional produce significantly differed in the average nitrate content. Organic produce had a lower nitrate content (1703 mg/kg), and conventional produce has a higher nitrate content (2637 mg/kg). Additionally, Woese et al. (1997) noted that conventionally cultivated or minerally fertilized vegetables typically contain far more nitrate than organically produced vegetables. The opposite situation, however, was observed in the study conducted by Gruszecka-Kosowska et al. (2017) where a higher nitrate level was found in organically leafy vegetables than the conventional one. Vegetables included are beet leaves, spinach broccoli, lettuce, kale, celery leaves, chive, and dill. The same goes with the result of Lima et al. (2009), where organically cultivated plants such as Chinese cabbage and maize have higher nitrate levels than the conventional ones. Even so, this study added to some existing results of conventionally grown vegetables having high nitrates than organically cultivated vegetables.

In addition, the result of the study showed a nitrate variation with the family being the *Brassicaceae* (Napa cabbage, Rape leaves, Bok Choy, A-Choy, Pak Choy) and *Amaranthaceae* (Spinach, Amaranth) – the families with the highest average levels. As Santamaria (2006) noted, plant families such as *Brassicaceae, Chenopodiaceae, and Amaranthaceae*; in addition to these are *Asteraceae*, and *Apiaceae* are usually the ones with the highest nitrate content among vegetables. In this research, this tendency was confirmed.

Differences observed in the results of cultivation practices among plant species could be attributed to fertilization practices along with the quantity and quality of chemical and biological fertilizers used. The rate, form, and timing of fertilizer applications all influence the uptake of nitrate by plants. According to Nemade and Attarde (2014), nitrogen-rich organic fertilizers can produce lower nitrate contents, but they can also contribute to significant nitrate accumulations when mineralization circumstances are optimal. This could explain why organic Amaranth and Napa cabbage have higher nitrate levels compared to their counterparts. Narayana and Sunil (2009) also claimed that the use of organic fertilizer such as compost with gradual or moderate available nitrogen explains the generally observed lower nitrate accumulation in organic vegetables. In addition, several authors reported various factors that determine nitrate contents in vegetable plants. Leafy vegetables with a shorter growing season and affected by unfavorable light conditions could impact the ability to accumulate nitrates in leaves. This statement might consider why Pak Choy which grows in a shorter period has the highest nitrate content for organic and conventional cultivation. Various factors determine nitrate contents in vegetables. Such factors include genetic factors which are responsible for 10% nitrate uptake in vegetables, cultivation period for 15%, soil conditions for 20%, fertilizing for 30%, and climatic conditions for 25%. This conclusion was stated by Sady (2000).

4.2 Exposure Assessment

4.2.1 Ingestion rate and body weight

Male and female average ingestion rates of consuming vegetables were gathered to calculate the DI of nitrate. Due to large uncertainty and availability, the parameters were considered as a distribution under the Monte Carlo simulation. As mentioned above Ingestion rate (IR) was fit to Lognormal distribution and Normal distribution for body weight. However, after fitted into distribution, the values of IR for both groups were the same. The table below shows the value of ingestion rate and body weight for both males and females.

Table 5. Values of IR and BW after fitted into distribution

Target Population	Ingestion Rate	Body Weight (BW)
Male	LN (9.81, 1.22)	N (72.30, 18.16)
Female	LN (9.81, 1.22)	N (58.40, 17.16)

4.2.2 Daily Intake of nitrate

In this study, estimated daily intake of both male and female were calculated using the equation

$$\mathrm{DI} = (\mathrm{Cveg} \times \mathrm{IR} \times \mathrm{CF})/\mathrm{BW}$$

where Cveg is vegetable concentration; IR is ingestion rate, CF is a conversion factor, and BW is body weight. All the values of these parameters were shown in chapter 3 of this study. Unit for DI is mg/kg/day. DI will be later used in characterizing risks.

As a result, consumption of conventionally cultivated leafy vegetables was linked to an average daily intake of nitrate of 0.36 mg/kg/day by the male group. Meanwhile, the estimated average daily intake of the female population is 0.45 mg/kg/day. On the other hand, the 97.5th percentile for males and females is 1.32 and 1.73, respectively. These results show that in terms of consuming leafy vegetables grown conventionally, the level of nitrate ingested by females daily is significantly higher than males. The same findings were found with the consumption of organic leafy vegetables, where the female group generated an average value of 0.57 mg/kg/day of nitrate. The male group, on the other hand, had an average DI of 0.47 mg/kg/day. The value generated for the 97.5th percentile of males and females respectively is 2.83 and 3.56 mg/kg/day. Findings from both organic and conventional farming show that females have a large daily intake of nitrate linked to the consumption of vegetables. As observed on the sensitivity chart of every calculation for DI, vegetable concentration contributes the most to the estimated DI. Between the target groups, females consume more vegetables than males. The reason for this finding is that females tend to consume more vegetables as part of their daily diet, thus causing them to ingest more nitrate. However, between the two farming practices, the highest value of DI falls on organic farming. Even so, the estimated daily intake of males and females doesn't exceed the Acceptable Daily Intake (ADI) of 3.7 mg/kg/day. Table and figures below show the percentile values of DI and graphs showing every value generated.

Table 6. Percentile values of DI

Percentiles	**2.5%-tile**	**25%-tile**	**50%-tile**	**75%-tile**	**97.5%-tile**
Conventional					
***male**	0.01	0.13	0.26	0.46	1.32
***female**	0.13	0.15	0.32	0.58	1.73
Organic					
***male**	0.02	0.07	0.16	0.41	2.83
***female**	0.03	0.09	0.20	0.49	3.56

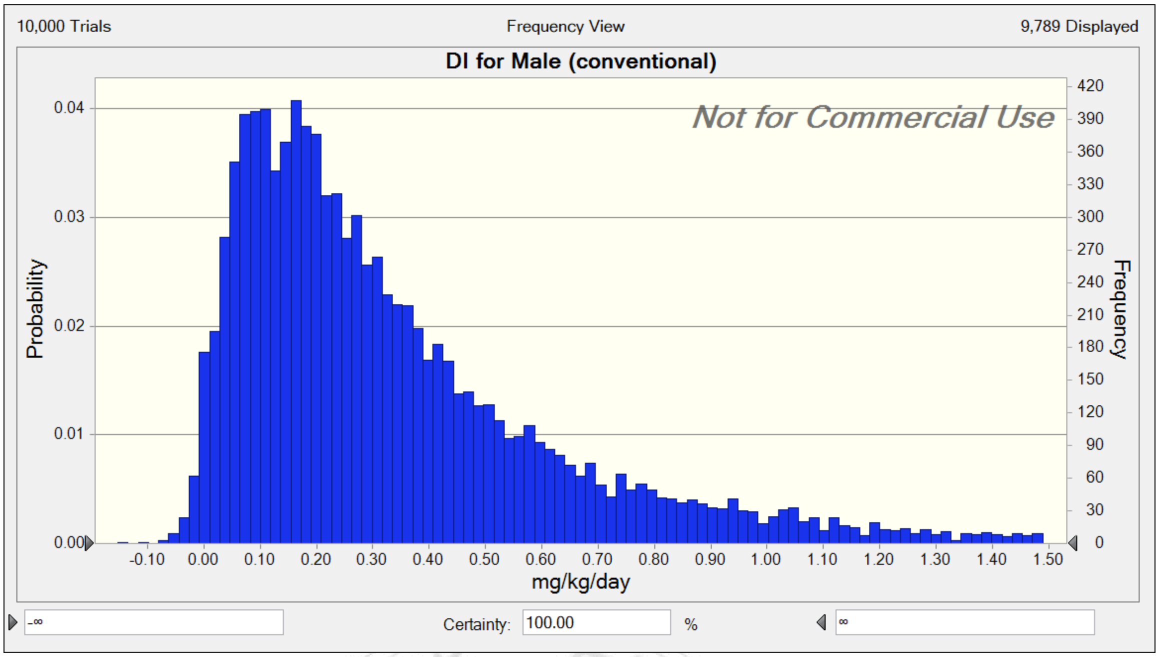

Figure 2. DI for Male (conventional)

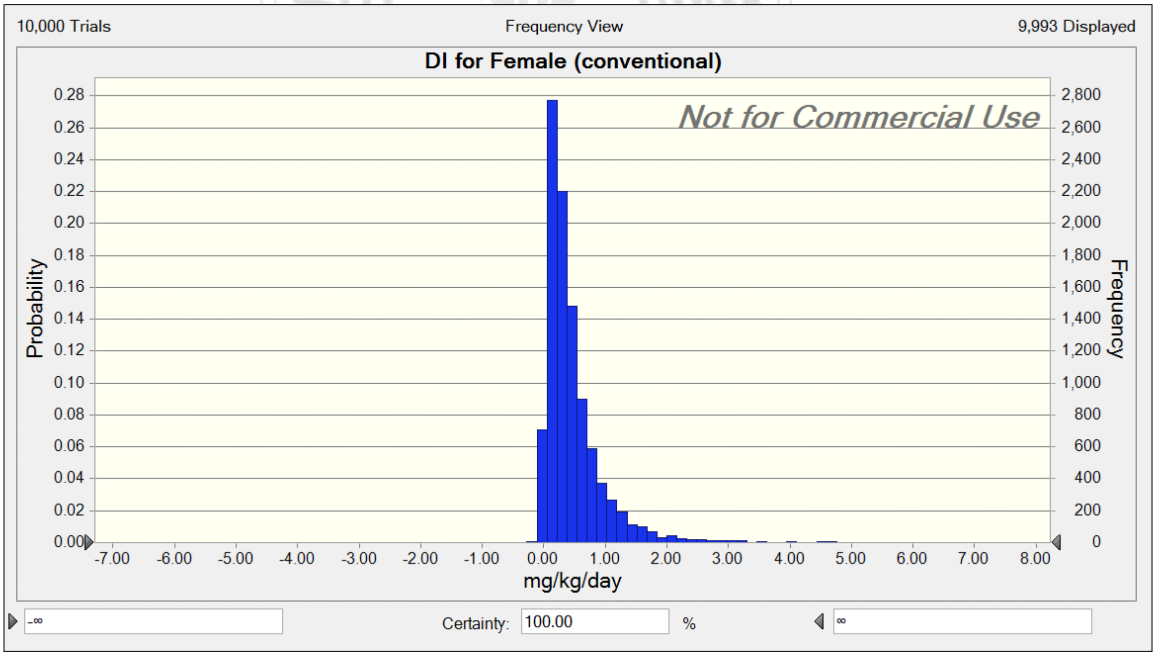

Figure 3. DI for Female (conventional)

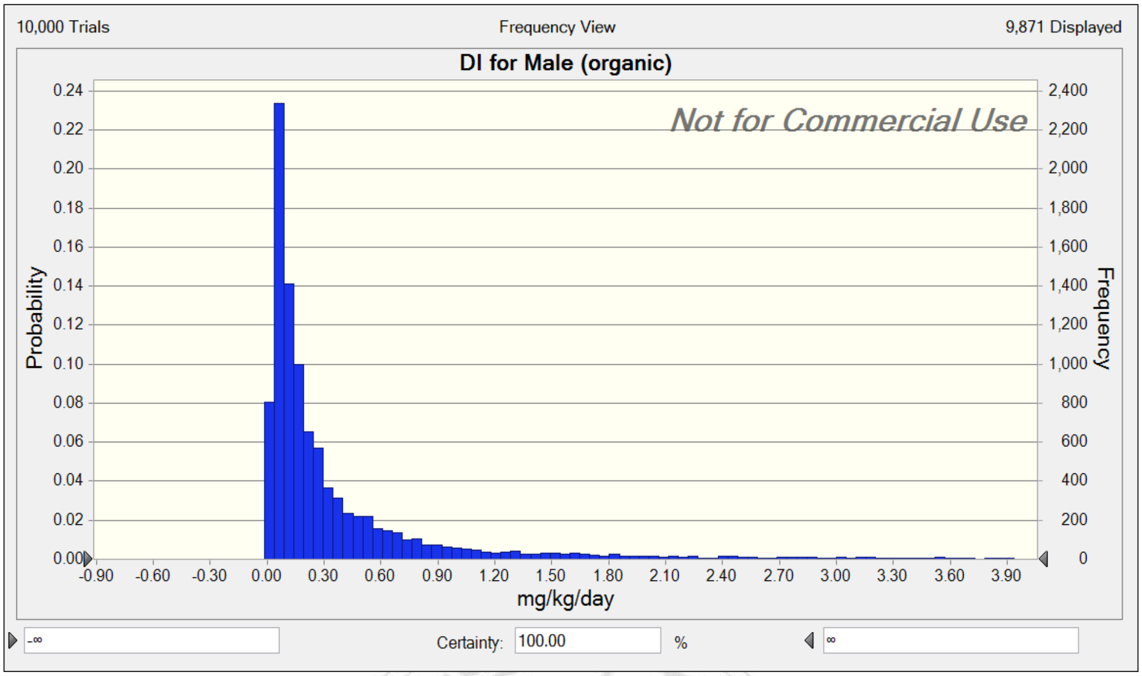

Figure 4. DI for Male (organic)

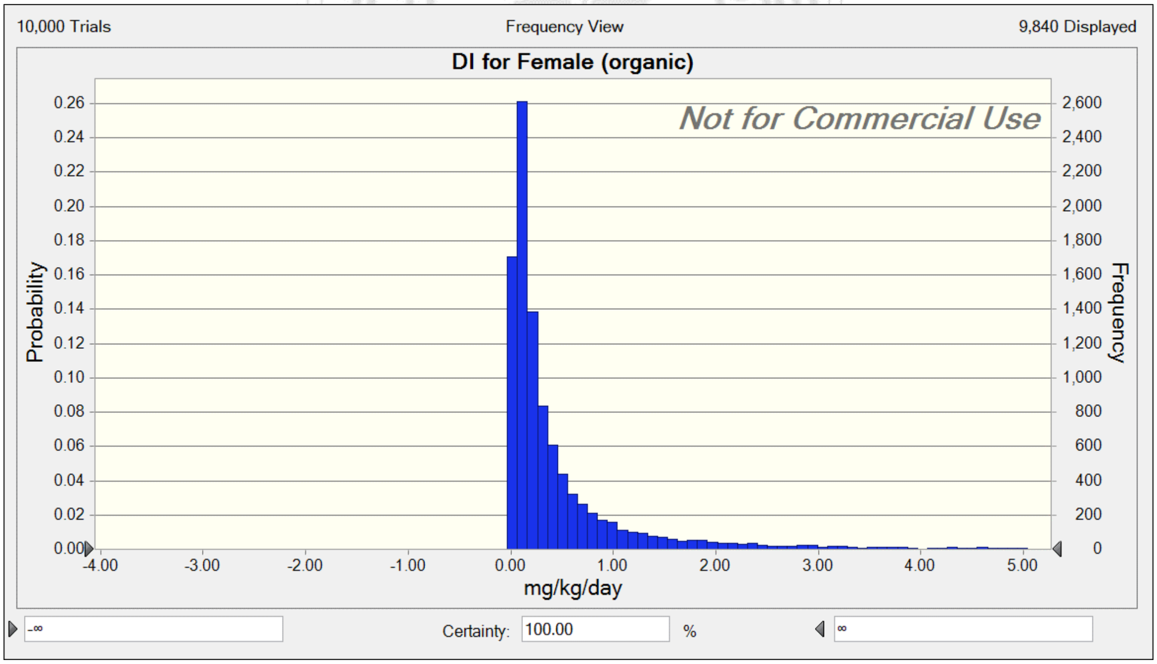

Figure 5. DI for Female (organic)

4.2.3 Sensitivity Analysis

All the sensitivity charts show that among all parameters, Cveg contributes the most to the results of DI for both groups. Followed by the IR, however, it can be suggested for both groups to increase in weight.

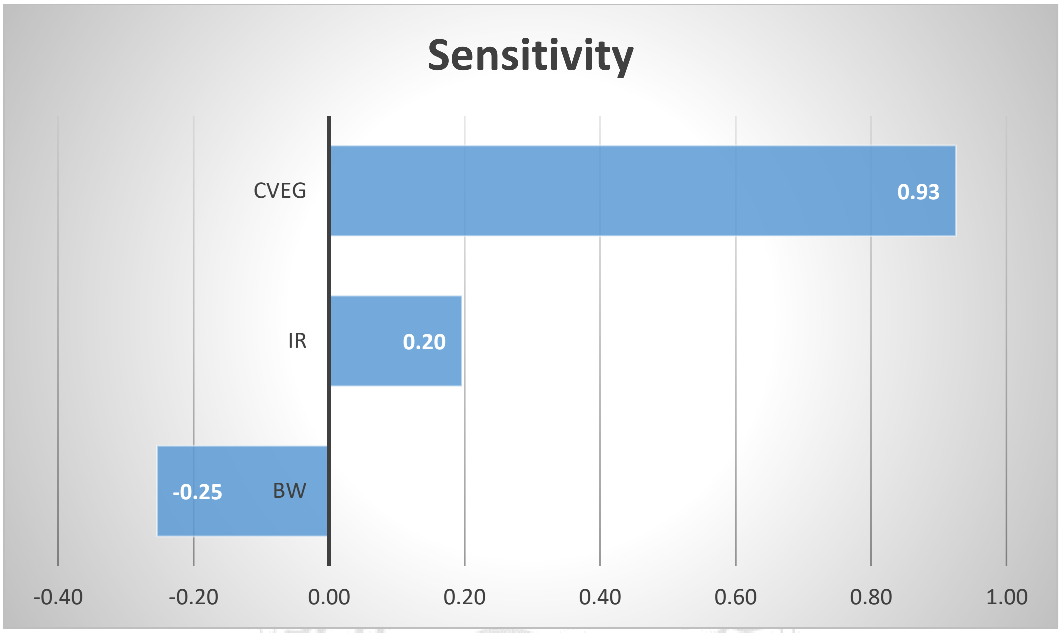

Figure 6. Sensitivity Chart for Male (conventional)

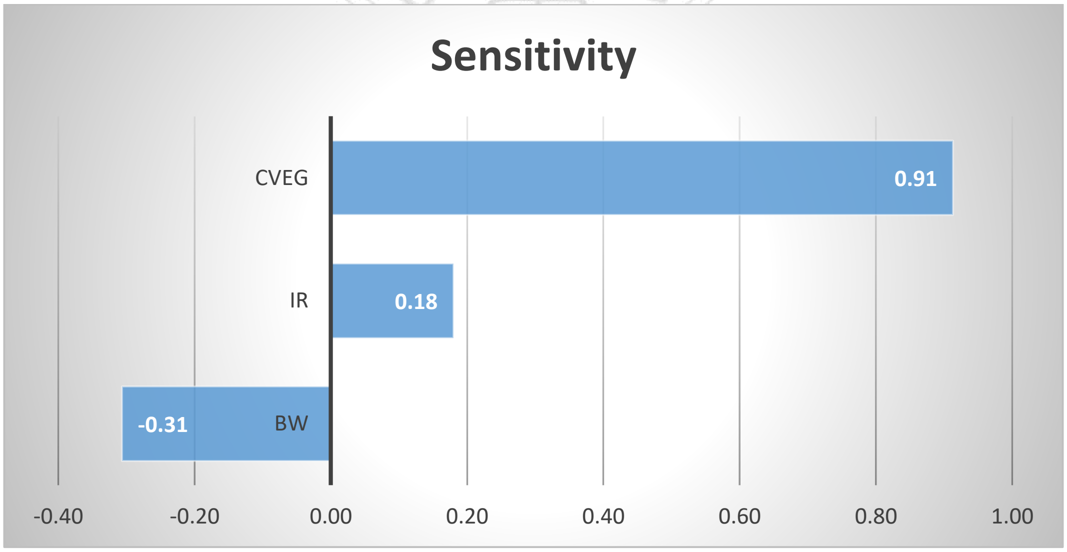

Figure 7. Sensitivity Chart for Female (conventional)

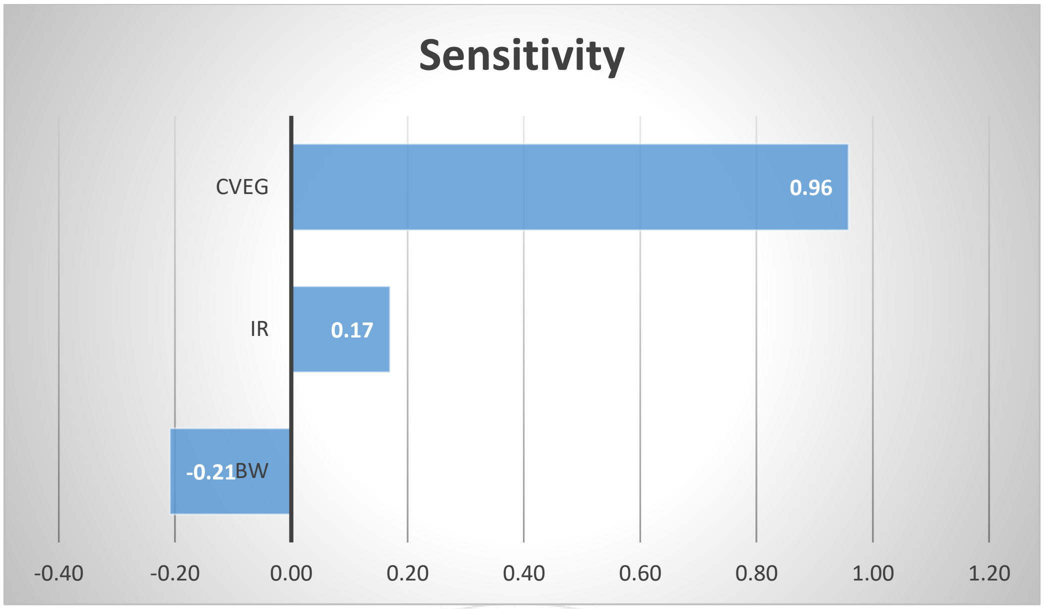

Figure 8. Sensitivity Chart for Male (organic)

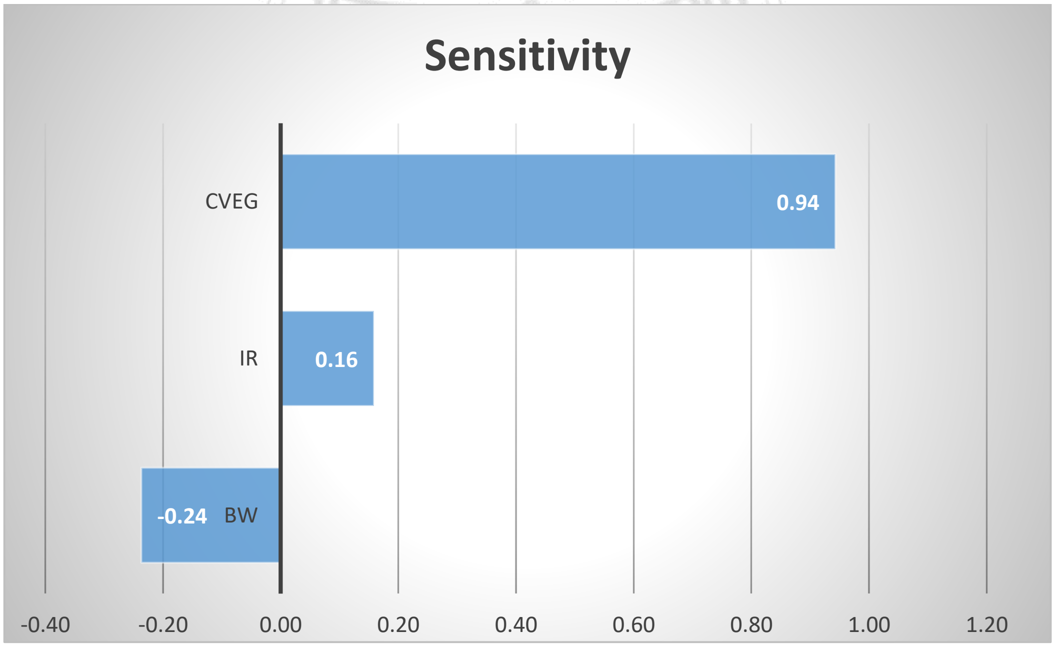

Figure 9. Sensitivity Chart for Female (organic)

4.3 Risk Characterization

4.3.1 Non-carcinogenic risk

Nitrate was reported by IRIS as non-carcinogenic, thus defining non-carcinogenic risk on the health of adult males and females here in Taiwan, posed by exposure to nitrate, the Hazard Quotient was calculated. The equation used is

$$HQ = DI / RfD$$

where RfD has the value of 1.6 mg/kg/day as proposed by USEPA, while the DI is provided above. The acceptable limit of HQ set by USEPA is 1. Lower than 1 may not pose any risk, however, greater than 1 may pose risk to human health. The results of the calculation are shown in the table below.

Table 7. Mean and 97.5%-tile of HQ

	DI (mg/kg)/day		**HQ**	
	Mean	**97.5%-tile**	**Mean**	**97.5%-tile**
Conventional				
***male**	0.36	1.32	0.23	0.82
***female**	0.45	1.73	0.28	1.08
Organic				
***male**	0.47	2.83	0.30	1.77
***female**	0.57	3.56	0.36	2.23

Based on the table above, values of the average-case scenario and worst-case scenario were calculated in identifying the non-carcinogenic risk of nitrate intake. It is shown that the mean value of HQ for males and females in conventional farming was 0.23 and 0.28, respectively. As per the result of organic farming, males have a value of 0.30, and female has 0.36. Therefore, the result indicates that the “average” non-carcinogenic risk for both groups can be considered acceptable. However, there is a significant difference between the mean value and the 97.5%-tile.

Based on conventional farming, the following results show that males, with a daily intake of 1.32 (mg/kg)/day, generated a result of 0.82, which is <1. By contrast, the female group has an HQ value of 1.08 with a nitrate intake of 1.73 (mg/kg)/day. This shows that the female group has a value significantly higher than the acceptable level of risk, therefore considered "unacceptable". Hence, associated with the consumption of leafy vegetables grown under conventional farming, females are more likely to experience adverse effects of nitrate intake than the male population.

Results under organic farming indicate that all predicted values of 97.5th percentiles of HQ are greater than 1, indicating the possibility of non-carcinogenic risks for both groups. The obtained HQ values for males and females are 1.77 and 2.23, respectively. However, it is evident from the above results, that for both organic and conventional farming, females are more subject to risk than their male counterparts. It is possible that the ingestion rate of vegetables may affect this result, as females are known to consume a lot of leafy vegetables every day. Hence, females should reduce the consumption of vegetables to lessen the intake of nitrate.

The Monte Carlo simulation results show that organically grown leafy vegetables can pose a health risk compared to conventionally grown vegetables. Below are the corresponding graphs for HQ calculations.

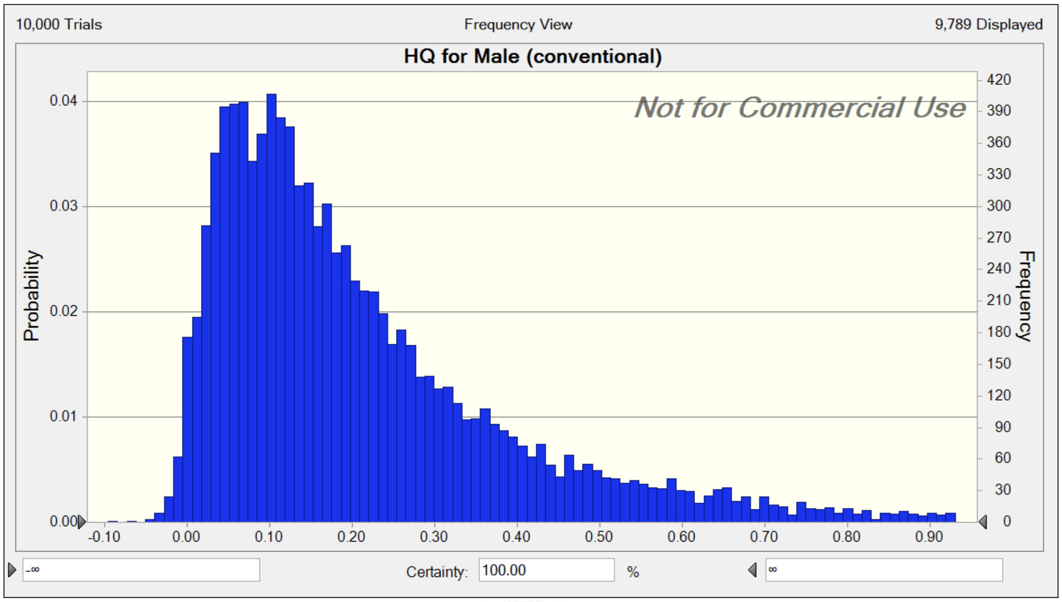

Figure 10. HQ for Male (conventional)

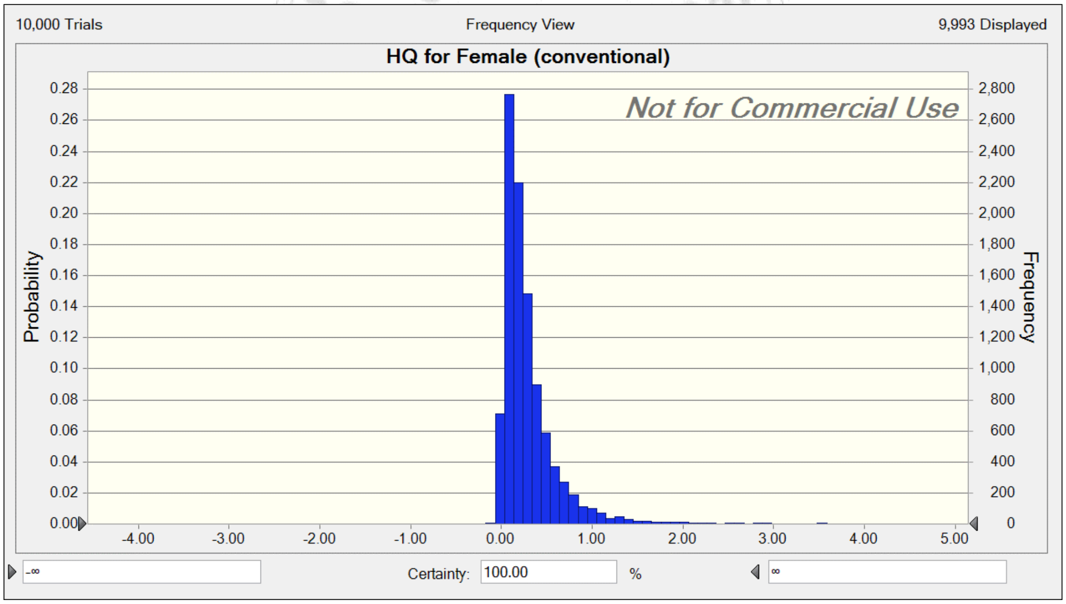

Figure 11. HQ for Female (conventional)

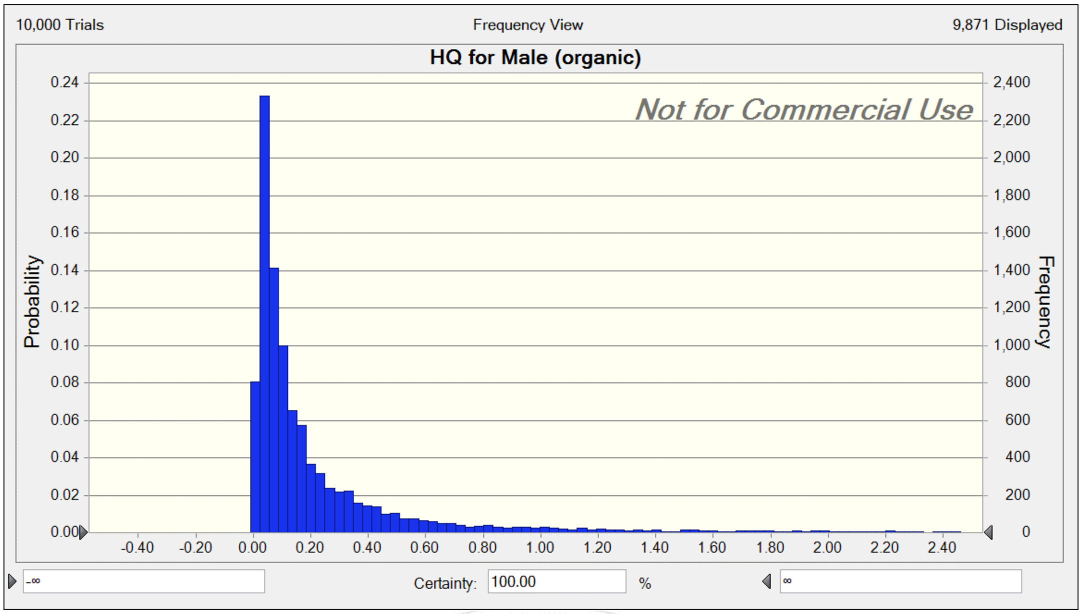

Figure 12. HQ for Male (organic)

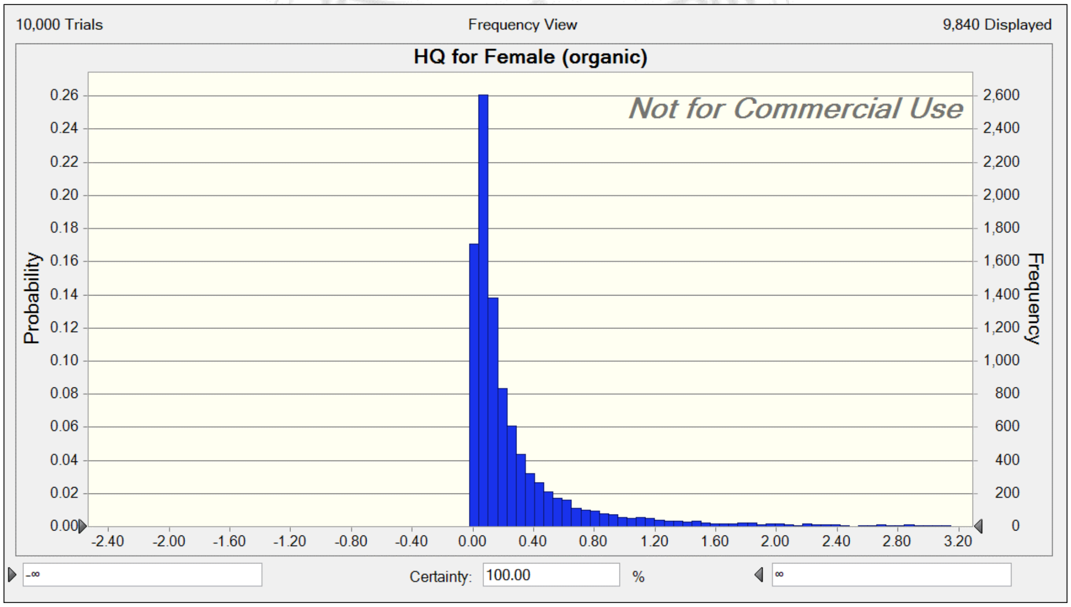

Figure 13. HQ for Female (organic)

4.3.2 Carcinogenic risk

Though nitrate is considered non-carcinogenic by USEPA, it might still possibly cause a potential risk to human health, associated with some factors linked to it. Thus, to finally characterize the carcinogenic risk that nitrate may pose, TR was calculated. The equation used in calculating TR is

$$TR = DI \times SF$$

In the absence of a slope factor for nitrate ingestion established by the USEPA, the existing value of the slope factor from Duvva et al. (2021) was used in calculating the TR in this study, which is 1×10^{-5} (kg*day)/mg. The table below shows the mean value and 97.5th percentile of TR based on the results.

Table 8. Mean and 97.5%-tile of TR

	TARGET RISK	
	mean	**97.5%-tile**
Conventional		
***male**	3.64×10^{-6}	1.32×10^{-5}
***female**	4.54×10^{-6}	1.73×10^{-5}
Organic		
***male**	4.74×10^{-6}	2.83×10^{-5}
***female**	5.73×10^{-6}	3.56×10^{-5}

Based on the results, all the figures obtained by males and females under conventional farming and organic farming were $>1\times10^{-6}$. Both the mean value and the 97.5%-tile have values above the acceptable level. Accordingly, it can be concluded that the carcinogenic risks associated with nitrate through the consumption of leafy vegetables grown through organic farming and conventional farming are considered acceptable. This may pose a cancer risk to certain populations. Below are the graphs of TR calculations from the Crystal ball.

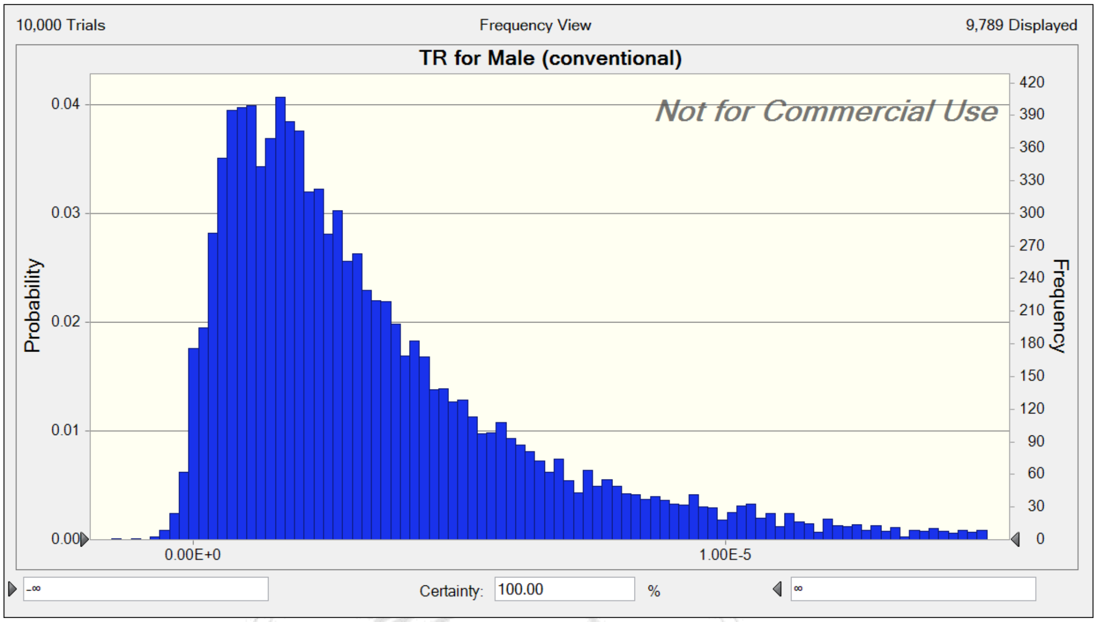

Figure 14. TR for Male (conventional)

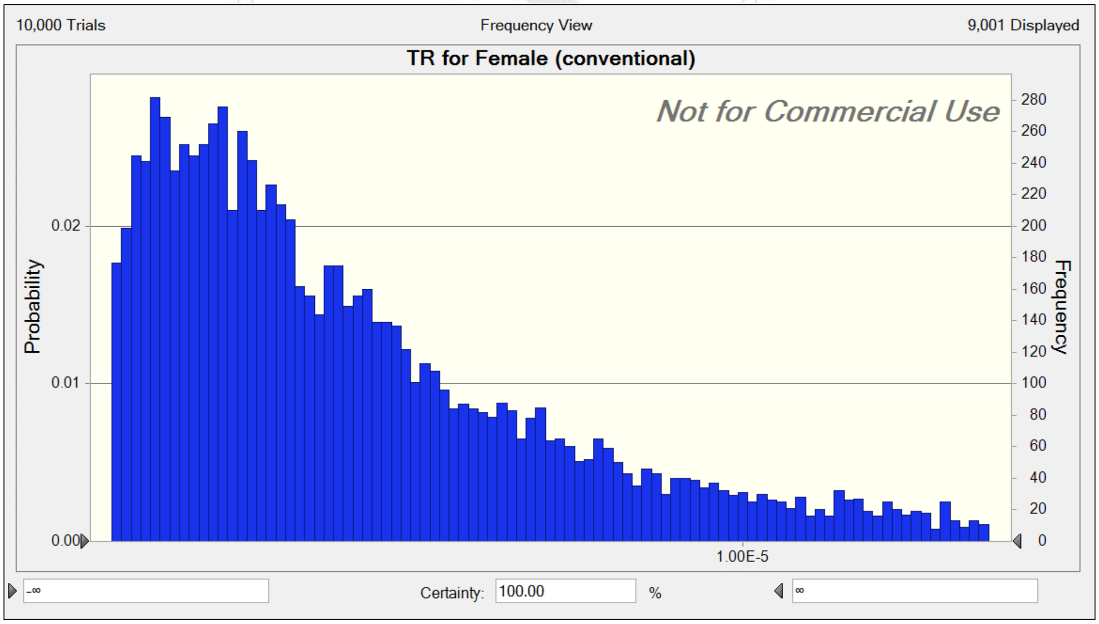

Figure 15. TR for Female (conventional)

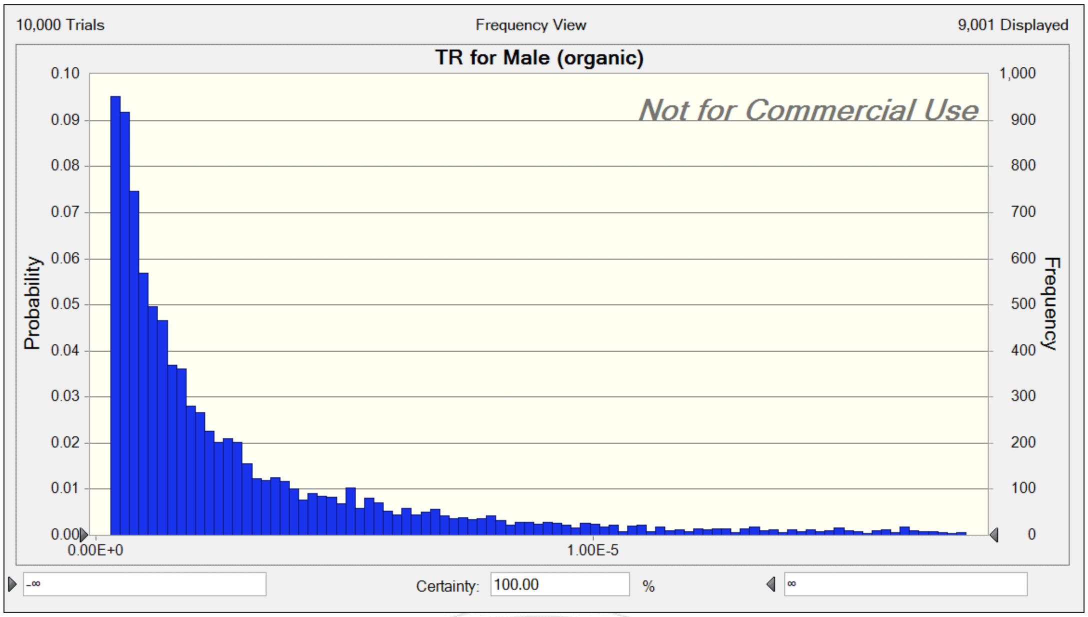

Figure 16. TR for Male (organic)

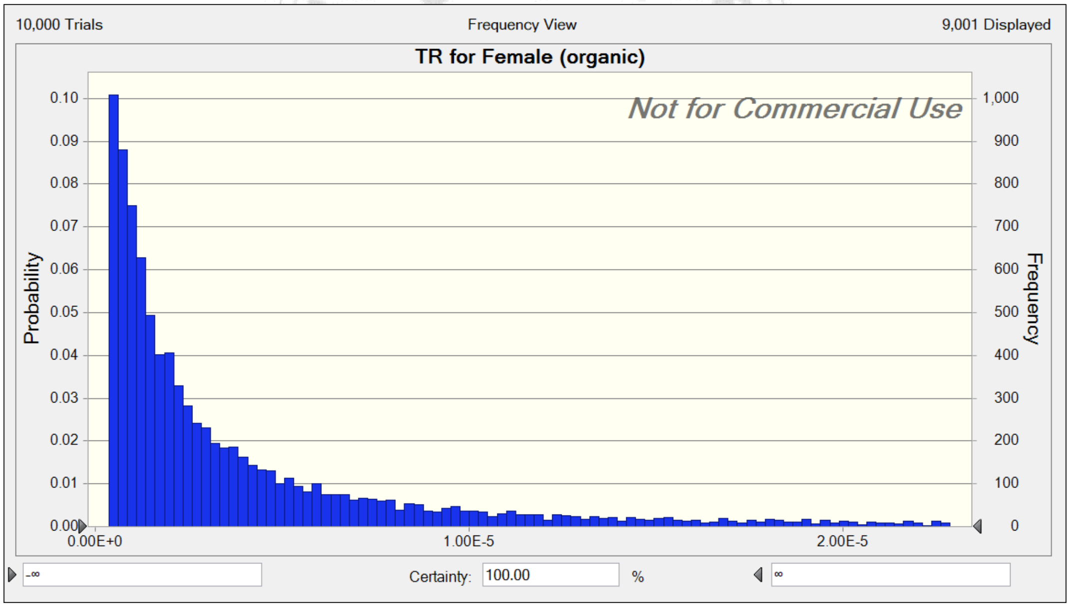

Figure 17. TR for Female (organic)

CHAPTER 5 CONCLUSIONS AND RECOMMENDATIONS

According to the results of this research, the nitrate concentrations of organic vegetables and conventionally grown leafy vegetables are significantly different. The geometric mean of organically grown vegetables was 996.02 mg/kg, while that of conventionally grown vegetables was 2497.97mg/kg. It is evident that conventionally grown leafy vegetables accumulate higher amounts of nitrates. The highest daily intakes of nitrates were obtained from the consumption of organic leafy vegetables by the female group, with a value of 3.56 mg/kg/day. However, DI results for both groups do not exceed the ADI of 3.7 mg/kg per day. Due to their large uncertainty and variability, the parameters were considered as distribution and the risk was calculated by Monte Carlo simulation using the Oracle Crystal Ball software. It is found that for non-carcinogenic risk, the mean values of HQ for both males and females associated with the consumption of vegetables produced under both farming practices are considered "acceptable". Yet, according to the results of the 97.5%-tile, results under organic farming are above the acceptable level, with the male group having a value of 1.77 and females having a value of 2.23. With conventionally grown vegetables, the male group generated a value of 0.82 while the female group generated a value of 1.08. Even though the HQ with the female group is >1, it is still lower than the organic results. Consequently, organically grown vegetables may pose a health risk to humans, especially women. As per the results of TR, the carcinogenic risk of nitrate through consumption of leafy vegetables can be considered as an acceptable risk.

Additionally, it is shown in the study that between the male and female groups, females are more likely to be at risk. This is due to the higher consumption rate of vegetables by females which is 309.12 g/day than males, which is 303.76 g/day. Although the consumption of leafy vegetables is widely promoted due to its numerous benefits to human health, it is still recommended that females should limit their intake of vegetables. This is to avoid high exposure to nitrate.

Results from this study could be used to set local nitrate intake standards in Taiwan. The current study was limited to adult males and females, so it is recommended that future research includes other groups, such as children or infants. Further experiments involving more kinds of leafy vegetables and other types of vegetables are suggested in future research. In addition, the study recommends using the same number of samples for subsequent experiments.

REFERENCES

Abo Bakr TM, El-Iraqui SM, Huissen MH. Nitrate and Nitrite Contents of Some Fresh and Processed Egyptian Vegetables. Food Chemistry. 1986; 19:265-275.

Agostini, F.; Tei, F.; Silgram, M.; Farneselli, M.; Benincasa, P.; Aller, M. Decreasing nitrate leaching in vegetable crops with better N management. In Genetic Engineering, Biofertilisation, Soil Quality and Organic Farming; Springer: Berlin, Germany, 2010; pp. 147–200.

Aires A, Carvalho R, Rosa EAS, and Saavedra MJ. 2013. Effects of agriculture production system on nitrate and nitrite accumulation on baby—leaf salads. Food Sci Nutr 1(1):3–7.

Basker, D. (1992) Comparison of taste quality between organically and conventionally grown fruits and vegetables. American Journal of Alternative Agriculture. 7:129-136.

Bazzano, L.A., Li, T.Y., Joshipura, K.J. & HU, F.B. (2008): Intake of fruit, vegetables, and fruit juices and risk of diabetes in women. Diabetes Care, 31, 1311–1317.

Bottex B, Dorne JLCM, Carlander D, et al. (2008) RiskBenefit Health Assessment of Food-Food Fortification and Nitrate in Vegetables. Trends Food Sci Technol 19: 113-119.

Brkić, D., Bošnir, J., Bevardi, M., Bošković, A. G., Miloš, S., Lasić, D., ... & Trstenjak, N. U. (2017). Nitrate in leafy green vegetables and estimated intake. *African Journal of Traditional, Complementary and Alternative Medicines*, *14*(3), 31-41.

Carlström, M., Persson, A. E. G., Larsson, E., Hezel, M., Scheffer, P. G., Teerlink, T., & Lundberg, J. O. (2011). Dietary nitrate attenuates oxidative stress, prevents cardiac and renal injuries, and reduces blood pressure in salt-induced hypertension. *Cardiovascular research*, *89*(3), 574-585.

Chan TY. Vegetable-borne nitrate and nitrite and the risk of methaemoglobinaemia. Toxicology letters. 2011; 200:107-108.

Council of Europe, Nitrates and Nitrites in Foodstuffs. Strasbourg, France (1993).

Domagala-Swiatkiewicz, I. (2013). Comparing nutritional content of fruits, vegetables and juices from organic and conventional crops. *CAB Reviews*.

Duncan, C., Dougall, H., Johnston, P., Green, S., Brogan, R., Leifert, C., Smith, L., Golden and M. and Benjamin, N. 1995. Chemical generation of nitric oxide in the mouth from the enterosalivary circulation of dietary nitrate. Nature Medicine, 1, 546 - 551.

Duncan, C., Li, H., Dykhuizen, R., Frazer, R., Johnston, P. & MacKnight, G. (1997): Protection against oral and gastrointestinal diseases: Importance of dietary nitrate intake, oral nitrate reduction and enterosalivary nitrate circulation. Comp. Biochem. Physiol., 118 A, 939–948.

Duvva, L. K., Panga, K. K., Dhakate, R., & Himabindu, V. (2022). Health risk assessment of nitrate and fluoride toxicity in groundwater contamination in the semi-arid area of Medchal, South India. *Applied Water Science*, *12*(1), 1-21.

EC (European Commission). 1992. Opinion on nitrate and nitrite. Reports of the Scientific Committee for Food (SCF) 26th Series, 21-28. Available at URL: http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_26.pdf

EC (European Commission). 1997. Opinion on nitrate and nitrite. Reports of the Scientific Committee for Food (SCF) 38th Series, 1-33. Available at URL: http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_38.pdf

EFSA (2008): Nitrate in vegetables. Scientific opinion of the panel on contaminants in the food chain. EFSA Journal, 689, 1e79, [cited 2011 Feb 10]. Available from: http://www.efsa.europa.eu/EFSA/Scientific_Opinion/ contam_ej_689_nitrate_en.pdf

FAO/WHO (Food and Agriculture Organisation of the United Nations/World Health Organization). 2003a. Nitrate (and potential endogenous formation of N-nitroso compounds). WHO Food Additive series 50, Geneva: World Health Organisation. Available at URL: http://www.inchem.org/documents/jecfa/jecmono/v50je06.htm

Gangolli, S.D., Van den Brandt, P.A., Feron, V.J., Janzowsky, C., Koeman, J.H., Speijers, G.J.A., Spiegelhalder, B., Walker, R. & Wishnok, J.S. (1994): Assessment of nitrate, nitrite and N-nitroso compounds. Eur. J. Pharmacol. Environ. Toxicol. Pharmacol. Section, 292, 1–38.

Gonzalez, M.C.M., M. J. Martınez-Tome, and M.E.T. Isasa. 2010. Nitrate and nitrite content in organically cultivated vegetables. Food Addit. Contam. 3:19–29.

Gorenjak, A.H., Cencic, A. Nitrate in vegetables and their impact on human health. A review. *Acta Alimentaria. 2013;42(2):158-172.*

Gruszecka-Kosowska, A., & Baran, A. (2017). Concentration and health risk assessment of nitrates in vegetables from conventional and organic farming. *Human and ecological risk assessment: an international journal*, *23*(4), 727-740.

Guadagnin, S.G., Rath, S. & Reyes, F.G.R. (2005): Evaluation of the nitrate content in leaf vegetables produced through different agricultural systems. Fd Addit. Contam., 22, 1203–1208.

Hord NG, Tang Y, Bryan NS. Food sources of nitrates and nitrites: the physiologic context for potential health benefits. American Journal of Clinical Nutrition. 2009; 90:1-10.

Hyde ER, Andrade F, Vaksman Z, Parthasarathy K, et al. Metagenotic analysis of nitrate-reducing bacteria in the oral cavity: implications for nitric oxide homeostasis. PLoS One. 2014;9(3), e88645.

Hsu J, Arcot J, Lee NA. Nitrate and nitrite quantification from cured meat and vegetables and their estimated dietary intake in Australians. Food Chemistry. 2009; 115:334-339.

Human Health Fact Sheet. 2005. Argonne National Laboratory, EVS Chicago, Illinois. Knorr, D., and H. Vogtmann. "Quality and quality determination of ecologically grown foods." In Sustainable food systems, edited by D. Knorr, 352-381. Westport, Connecticut: AVI Publishing company, 1983.

Integrated Risk Information System (IRIS). U.S. Environmental Protection Agency Chemical Assessment Summary. National Center for Environmental Assessment. https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0076_summary.pdf#nameddest=rfd

Iammarino M, Di Taranto A, Cristino M. Monitoring of nitrites and nitrates levels in leafy vegetable (spinach and lettuce): a contribution to risk assessment. J Sci Food Agric 2014; 94:773-778.

Jakszyn, P., & González, C. A. (2006). Nitrosamine and related food intake and gastric and oesophageal cancer risk: a systematic review of the epidemiological evidence. *World journal of gastroenterology: WJG*, *12*(27), 4296.

Joossens, J.V., Hill, M.J., Elliott, P., Stamler, R., Stamler, J. & Lesaffre, E., Dyer, A., Nichols, R., Kesteloot, H. and on behalf of European Cancer Prevention (ECP) and the Intersalt Cooperative Research Group (1996): Dietary salt, nitrate and stomach cancer mortality in 24 countries. Int. J. Epidemiol., 25, 494–504.

Kim, J., H., Lee, S. S., Choi, B. Y., & Kim, M. K. (2007). Nitrate intake relative to antioxidant vitamin intake affects gastric cancer risk: a case-control study in Korea. *Nutrition and cancer*, *59*(2), 185-191.

Knobelock, L., Salna, B., Hogan, A., Postle, J. & Anderson, H. (2000): Blue babies and nitrate-contaminated well water. Environ. Health Perspect., 108, 675–678.

Larsen, F.J., Weitzberger, E., Lundberg, J.O. & Ekblom, B. (2007): Effects of dietary nitrate on oxygen cost during exercise. Acta Physiol. (Oxf), 191, 59–66.

Lima GPP, Lopes TVC, Rossetto MRM, et al. 2009. Nutritional composition, phenolic compounds, nitrate content in eatable vegetables obtained by conventional and certified organic grown culture subject to thermal treatment. Int J Food Sci Technol 44:1118–24.

Liu, C.W., Sung y., Chen B.C., Lai H.Y. Effects of nitrogen fertilizers on the growth and nitrate content of lettuce (*Lactuca sativa* L.). *Int. J. Environ. Res. Public Health* 2014;11,4427-4440.

Lundberg JO, Weitzberg E. Biology of nitrogen oxides in the gastrointestinal tract. Gut. 2013;62(4);616-29.

Lundberg, J.O., Weitzberg, E. Gladwin, M.T. 2008. The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. Nature Reviews Drug Discovery 7, 156-167.

Mensinga TT, Speijers GJA and Meulenbelt J, Health implications of exposure to environmental nitrogenous compounds. *Toxicol Rev* **22**:41–51 (2003).

Michaud DS, Mysliwiec PA, Aldoori W, Willett WC, Giovannucci E. Peptic Ulcer Disease and the Risk of Bladder Cancer in a Prospective Study of Male Health Professionals, Cancer Epidem. Biomar. 2004; 13:250- 254.

Narayana, B. and Sunil, K. (2009). A Spectrophotometric Method for the Determination of Nitrite and Nitrate, Eurasian J. Anal, 4: 204-214.

National Feeding Database (2017). Food and Drug Administration. National Institutes of Health. National Feeding Database (nhri.edu.tw)

Nemade, K., & Attarde, S. (2014). Development and validation of UV spectrophotometric method for simultaneous estimation of nitrate and nitrite in water, soil, drug and vegetable samples. *International Journal of Advanced Chemistry, 2*(2), 74-79. doi:http://dx.doi.org/10.14419/ijac.v2i2.2619

Nuñez MTG, Osburn WN, Hardin MD, Longnecker M, Garg HK, Bryan NS, Keeton JT. Survey of nitrate and nitrite contents of conventional and organic labeled raw vegetables available at retail in the United States. J Food Sci. 2015;80(5):C942-9.

Paxton, P. M., Curran, P., Bollen, K. A., Kirby, J., & Chen, F. (2001). Monte Carlo simulations in structural equation models. *Structural Equation Modeling*, *8*, 287-312.

Pragani, M. A., Desai, K. P., Morrone, D., Sidhu, M. S., & Boden, W. E. (2017). The role of nitrates in the management of stable ischemic heart disease: a review of the current evidence and guidelines. *Reviews in cardiovascular medicine*, *18*(1), 14-20.

Pussemier, L., Larondelle, Y., Van Peteghem, C., & Huyghebaert, A. (2006). Chemical safety of conventionally and organically produced foodstuffs: a tentative comparison under Belgian conditions. *Food control*, *17*(1), 14-21.

Qiu, H., Gui, H., Fang, P., & Li, G. (2021). Groundwater pollution and human health risk based on Monte Carlo simulation in a typical mining area in Northern Anhui Province, China. *International Journal of Coal Science & Technology*, *8*(5), 1118-1129.

Rembialkowska, E. 2007. Quality of plant products from organic agriculture. J. Sci. Food Agric. 87:2757–2762.

Santamaria P. Contributo degli ortaggi all’assunzione giornaliera di nitrato, nitrito e nitrosammine. *Industrie Alimentari* **36**:1329–1334 (1997).

Santamaria P, Elia A, Gonnella M. Changes in nitrate accumulation and growth of endive plants during the light period as affected by nitrogen level and form. *J Plant Nutr* **20**:1255–1266 (1997).

Santamaria P, Elia A, Serio F, Todaro E. A survey of nitrate and oxalate content in retail fresh vegetables. *J Sci Food Agric* **79**:1882–1888 (1999).

Santamaria P, Elia A, Gonnella M, Parente A, Serio F. Ways of reducing rocket salad nitrate content. *Acta Hortic* **548**:529–537 (2001).

Santamaria, P. 2006. Nitrate in vegetables: toxicity, content, intake and EC regulation (review). J. Sci. Food Agric. 86:10– 17.

SCF (Scientific Committee on Food), Assessment of dietary intake of nitrates by the population in the European Union, as a consequence of the consumption of vegetables, in *Reports on tasks for scientific cooperation: report of experts participating in Task 3.2.3,* ed by European Commission, Brussels, p 34 (1997).

Song P, Wu L, Guan W (2015). Dietary Nitrates, Nitrites, and Nitrosamines Intake and the Risk of Gastric Cancer: A Meta-Analysis. Nutrients, 7:9872- 9895.

Tamme T, Reinik M, Roasto M, Juhkam K, Tenno T, Kiis A. Nitrates and nitrites in vegetables and vegetablebased roducts and their intakes by the Estonian population. Food Additives and Contaminants. 2006; 23(4):355-361.

Triantafyllidis V, Papasavvas A, Hela D, Salahas G. Comparison of nitrate content in leafy vegetables conventionally and organically cultivated in Western Greece. *Journal of Environmental Protection and Ecology.* 2008;9(2):301-308.

Walker R. The metabolism of dietary nitrites and nitrates. *Biochem Soc Trans* **24**:780–785 (1996).

Woese, K., Lange, D., Boess, C. & Bögl, K.W. (1997): A comparison of organically and conventionally grown foods: Results of a review of the relevant literature. J. Sci. Fd Agric., 74, 281–293.

World Health Organization. 2008. WHO European Action Plan for Food and Nutrition 2007–2012. WHO, Copenhagen, Denmark

Zhong W, Hu C, Wang M. Nitrate and nitrite in vegetables from north China: Content and intake. Food Additives and Contaminants. 2002; 19(12):1125-1129.

APPENDIX

Appendix I. Monte Carlo simulation results

(a). Data of male group under conventional farming

Statistics	**DI**	**HQ**	**TR**	**BW**	**Cveg**	**IR**
Trials	10000	10000	10000	10000	10000	10000
Base Case	---	---	---	0.00	0.00	0.00
Mean	0.36	0.23	0.00	72.39	2,424.96	10.02
Median	0.26	0.16	0.00	72.61	1,878.61	9.79
Standard Deviation	0.40	0.25	0.00	18.23	2,194.82	2.01
Variance	0.16	0.06	0.00	332.25	4,817,239.89	4.04
Skewness	4.78	4.78	4.78	0.0023	2.75	0.6167
Kurtosis	59.89	59.89	59.89	3.08	19.02	3.64
Coeff. of Variation	1.09	1.09	1.09	0.2518	0.9051	0.2007
Minimum	-0.12	-0.07	0.00	4.04	-425.58	4.41
Maximum	9.96	6.22	0.00	152.83	33,406.56	19.18
Range Width	10.08	6.30	0.00	148.79	33,832.14	14.77
Mean Std. Error	0.00	0.00	0.00	0.18	21.95	0.02
Percentiles						
2.5%	0.01	0.01	1.05E-07	36.11	81.88	6.64
25%	0.13	0.08	1.32E-06	60.00	986.14	8.62
50%	0.26	0.16	2.60E-06	72.61	1878.57	9.79
75%	0.46	0.29	4.64E-06	84.68	3203.14	11.22
95.7%	1.32	0.82	1.32E-05	108.01	7955.87	14.48
Sensitivity Data						
Assumptions	**BW**	**Cveg**	**IR**			
	-0.25	0.93	0.20			

(b). Data of female group under conventional farming

Statistics	**DI**	**HQ**	**TR**	**BW**	**Cveg**	**IR**
Trials	10000	10000	10000	10000	10000	10000
Base Case	---	---	---	0.00	0.00	0.00
Mean	0.45	0.28	0.00	58.72	2398.08	10.01
Median	0.32	0.20	0.00	58.76	1838.59	9.83
Standard Deviation	0.58	0.36	0.00	17.04	2,176.71	1.99
Variance	0.34	0.13	0.00	290.28	4,738,059.13	3.95
Skewness	-7.94	-7.94	-7.94	-0.0146	2.58	0.6185
Kurtosis	505.30	505.30	505.30	2.94	17.14	3.91
Coeff. of Variation	1.28	1.28	1.28	0.2902	0.9077	0.1986
Minimum	-26.87	-16.79	0.00	-6.88	-404.04	4.58
Maximum	7.61	4.75	0.00	123.95	35,874.26	23.65
Range Width	34.48	21.55	0.00	130.83	36,278.30	19.07
Mean Std. Error	0.01	0.00	0.00	0.17	21.77	0.02
Percentiles						
2.5%	0.01	0.01	9.63E-08	25.12	55.21	6.66
25%	0.15	0.10	1.55E-06	47.16	949.38	8.62
50%	0.32	0.20	3.18E-06	58.76	1,838.51	9.83
75%	0.58	0.37	5.84E-06	70.22	3,186.21	11.22
95.7%	1.73	1.08	1.73E-05	91.73	8,022.47	14.41
Sensitivity Data						
Assumptions	**BW**	**Cveg**	**IR**			
	-0.31	0.91	0.18			

(c). Data of male group under organic farming

Statistics	**DI**	**HQ**	**TR**	**BW**	**Cveg**	**IR**
Trials	10000	10000	10000	10000	10000	10000
Base Case	---	---	---	0.00	0.00	0.00
Mean	0.47	0.30	0.00	72.10	3,045.49	10.02
Median	0.16	0.10	0.00	72.16	1,126.17	9.82
Standard Deviation	1.79	1.12	0.00	18.11	7,574.72	2.00
Variance	3.22	1.26	0.00	328.11	57,376,408.06	4.00
Skewness	42.28	42.28	42.28	0.0099	16.08	0.5552
Kurtosis	2,723.95	2,723.95	2,723.95	2.98	501.43	3.38
Coeff. of Variation	3.78	3.78	3.78	0.2512	2.49	0.1996
Minimum	0.01	0.01	0.00	6.53	148.49	4.97
Maximum	128.37	80.23	0.00	141.90	328,743.58	19.32
Range Width	128.36	80.22	0.00	135.37	328,595.09	14.35
Mean Std. Error	0.02	0.01	0.00	0.18	75.75	0.02
Percentiles						
2.5%	0.02	0.02	2.44E-07	36.39	197.64	6.62
25%	0.07	0.04	7.08E-07	59.73	515.97	8.61
50%	0.16	0.10	1.60E-06	72.16	1,126.16	9.82
75%	0.41	0.26	4.13E-06	84.34	2,802.02	11.23
95.7%	2.83	1.77	2.83E-05	108.05	18,278.71	14.49
Sensitivity Data						
Assumptions	**BW**	**Cveg**	**IR**			
BW	-0.21	0.96	0.17			

(d). Data of female group under organic farming

Statistics	**DI**	**HQ**	**TR**	**BW**	**Cveg**	**IR**
Trials	10000	10000	10000	10000	10000	10000
Base Case	---	---	---	0.00	0.00	0.00
Mean	0.57	0.36	0.00	58.73	3,018.55	10.00
Median	0.20	0.12	0.00	58.73	1,115.15	9.78
Standard Deviation	2.02	1.26	0.00	17.15	7,711.29	2.00
Variance	4.09	1.60	0.00	294.14	59,464,017.83	3.99
Skewness	9.97	9.97	9.97	-0.0213	15.64	0.5646
Kurtosis	850.12	850.12	850.12	3.02	458.54	3.48
Coeff. of Variation	3.53	3.53	3.53	0.2920	2.55	0.1997
Minimum	-79.98	-49.99	0.00	-3.01	149.16	4.56
Maximum	96.25	60.16	0.00	128.22	295,727.22	20.48
Range Width	176.24	110.15	0.00	131.23	295,578.07	15.92
Mean Std. Error	0.02	0.01	0.00	0.17	77.11	0.02
Percentiles						
2.5%	0.03	0.02	2.93E-07	25.06	201.70	6.58
25%	0.09	0.06	8.84E-07	47.22	513.48	8.60
50%	0.20	0.12	1.99E-06	58.73	1,115.01	9.78
75%	0.49	0.31	4.91E-06	70.47	2,725.62	11.22
95.7%	3.56	2.23	3.56E-05	92.03	17,734.15	14.48
Sensitivity Data						
Assumptions	**BW**	**Cveg**	**IR**			
	-0.24	0.94	0.16			