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蔬菜中硝酸鹽濃度之季節變異及其對台灣居民之人體健康風險評估

Seasonal Difference of Nitrate Level in Vegetables and Subsequent Human Health Risk Assessment for Taiwan Residents

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摘要

人體攝取硝酸鹽主要途徑為水及蔬菜，而藉由食用蔬菜攝入硝酸鹽，一般被認為是最主要的暴露途徑。在攝入硝酸鹽之前或之後，硝酸鹽可能轉化為具高鐵血紅蛋白亞硝酸鹽，並產生潛在的風險，進而對人類健康產生不利的影響。本研究探討於夏季及冬季收穫蔬菜，對總硝酸鹽濃度之影響，並進行因食用蔬菜而暴露於硝酸鹽的成年男性和女性之人體健康風險評估。研究對象鎖定在臺灣的成年(19-44 歲)男性與女性，計算其對因蔬菜攝食對硝酸鹽的每日攝入量 (*DI*) 。考量因子包括蔬菜中硝酸鹽濃度(C_{veg})、成年男性和女性的蔬菜攝食率 (*IR*) 和平均體重 (*BW*)。透過計算危害商數 (*HQ*)及標的終生風險(*TR*)值，以評估因攝食蔬菜攝入硝酸鹽所造成的致癌性和非致癌性潛在風險。

本研究中，夏季和冬季的 C_{veg} 平均值分別為 1,908.84 和 1, 490.24 mg/(kg*day)。夏季蔬菜部分，男性和女性 *DI* 值之 95% 信賴區間上限分別為 1.37 和 1.73 mg/(kg*day)；冬季蔬菜部分，男性和女性 *DI* 值之 95% 信賴區間上限則分別為 1.08 和 1.45 mg/(kg*day)。危害商數 *HQ* 部分，男性和女性攝食夏季蔬菜之模擬結果分別為 0.82 和 1.08；若考量攝食冬季生產之蔬菜，男性和女性之 *HQ* 值則分別為 0.67 和 0.91。研究結果顯示，除針對女性攝取夏季生產之蔬菜外，其餘所有 *HQ* 值之 95%信賴區間上限都低於 1，代表臺灣居民因攝食蔬菜而暴露於硝酸鹽之潛在非癌症風險落於可接受之範圍。另一方面，男性和女性之癌症風險值 *TR* 的 95%信賴區間上限值，於攝食夏季產蔬菜分別為 1.33 x10^{-5} 和 1.73 x10^{-5}，於攝食冬季產蔬菜則為 1.09 x10^{-5} 和 1.45 x10^{-5}。研究結果顯示，所有癌症風險 (*TR*) 值介於可忽略及可接受之間，而

攝食夏季產之蔬菜風險高於攝食冬季生產之蔬菜。另，本研究中不論考量攝食夏季或冬季生產之蔬菜，成年女性之 *DI*、*HQ* 和 *TR* 值皆高於男性。

由於蔬菜攝食為硝酸鹽攝取之主要來源，且其對人體健康危害有影響，因此該議題普遍受到公共衛生及政府相關管理機構之重視，此亦為進行本研究之主要原因及動機。

關鍵詞：硝酸鹽、蔬菜、風險評估、危害

ABSTRACT

Majority of nitrates are consumed through water and vegetables. In addition, the intake of nitrate through vegetable consumption is regarded as an extensive exposure route in humans. Before and/or after ingestion, the conversion of methaemoglobin-producing nitrite from nitrate develops a potential risk and is frequently associated with adverse effects on human health. An investigation was conducted to study the total nitrate concentration under the influence of harvesting time during summer and winter. The subsequent human health risk of nitrate exposure was then assessed for both adult male and female exposed to nitrate through vegetable consumption. Total exposure of nitrate from vegetable consumption was assessed using the data gathered from adult (19-44) male and female Taiwanese residents. The total daily intake (*DI*) values were determined by using the data of vegetable nitrate concentration (C_{veg}), ingestion rate of vegetable (*IR*), and the body weight (*BW*) of both adult male and female residents. The potential non-carcinogenic and carcinogenic risks of nitrate exposure from vegetables consumption were evaluated by the characterization of hazard quotient (*HQ*) values and the target lifetime risk (*TR*) values.

In the present result, mean values of C_{veg} for both summer and winter are 1,908.84 and 1,490.24 mg/(kg*day), respectively. The upper 95% confidence limit of *DI* values during summer time for male and female were 1.37 and 1.73 mg/(kg*day), respectively. On the other hand, *DI* values during winter time for male and female were respectively 1.08 and 1.45 mg/(kg*day). Simulation results showed that for the upper 95% confidence limit of *HQ* values during summer time for male and female were 0.82 and 1.08, respectively. In addition, *HQ* values for winter time for male and female residents were 0.67 and 0.91. In the present result, all predicted upper 95% confidence limit of *HQs* were lower than 1, indicating the potential non-cancer risk of nitrate associated with vegetable consumption for Taiwan residents was acceptable except for female during summer time. For cancer risk, result demonstrate that the upper 95% confidence limit of *TR* for male and female was respectively 1.33 x10^{-5} and 1.73 x10^{-5} during summer, and was 1.09 x10^{-5} and 1.45 x10^{-5} for winter time, respectively. This results showed that all cancer risk (*TR*) value ranged between the negligible level (10^{-6}) and acceptable level (10^{-4}). Therefore, the cancer risk of nitrate associated with vegetables consumption should be noticed. The cancer risk for vegetables in summer season was higher than that in winter season. In addition, the *DI*, *HQ*, and *TR* values for adult female were higher than those for male, both for summer and winter season vegetable consumption.

Since that vegetable consumption became a major source for dietary nitrate intake and often associated with harmful effects on human health, long-term adverse health effects gathered interest to public health providers and even government regulators, hence, this study was conducted.

Keywords: nitrate, vegetables, risk assessment, hazard

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CHAPTER 1 PREFACE

1.1 INTRODUCTION

Nitrates are naturally occurring plant constituents that are eaten along with vegetables and fruits. Despite the undeniable contribution of nitrate exposure to human health, end consumers and other health risk enthusiasts are worried about the risk posed by nitrate exposure from vegetable intake, which may compromise product quality and safety, recognizing that the Nitrates found in vegetable and water, including other food source may show a warning for health. Due to the high concentration of this chemical compound, it has been the subject of several reports and studies to assess its possible danger to human health. New data on nitrate and nitrite had shown limited potential risk, and as an outcome, the advantage of the assessment was analyzed and it was found out that the possible interest can highly exceed any upcoming risk using exact measures and course of regulation. It is well-known that the presence of nitrates is made in the soil by the different factors with the help of microorganisms, hence, Nitrates has been accessible for the development of plants. Several factors were considered when assessing the nitrate content of vegetables, but the greater risk is associated with its transformation to nitrite that produce methaemoglobin under the process of ingestion. In certain cases, nitrate is a functional food component that prevents pathogens by acting as an effective antimicrobial. Furthermore, Nitrite has been linked to a number of long-term negative health impacts, piquing the interest of public health officials and government authorities. In the soil, nitrate is a naturally occurring molecule. For healthy plant growth, a sufficient supply of this chemical compound is needed and the majority of nitrogen absorbed by plants is in the form of nitrate. (Bryan et al, 2017). Plants nitrate content is also dictated by their genes and the level of nitrate that is present in the soil. When fertilizer is applied in high amount than the crop's capacity to utilize, nitrate can build up, especially if another vital nutrient is low. At low concentrations, Nitrate is generally considered to be harmless. Nitrite, on the contrary, is a responsive particle that can nitrosate other molecules in the stomach's extreme ph, such as proteins, amines, and amides. Although nitrites are rarely present in the environment, the majority of human exposure comes from ingested nitrate, which can be converted to nitrite by the bacteria present in the saliva. (Tannenbaum, 1976). The use of dietary nitrate as a bio - active nitric oxide donor has attracted interest due to its potential cardio protective and ergogenic qualities. According to a recent study, oral nitrate intake causes both immediate and persistent reductions in resting blood pressure, as well as increased revascularization in chronic ischemia. van der Avoort et al. (2020) added that nitrate has been shown to improve fitness and/or functional efficiency.

In some studies, Nitrate and Nitrite naturally occurs in food. These chemical compounds are largely available in nature. Having high amounts of these compounds in soil, vegetables, and water is necessary for the food chain of all living organisms and Nitrates are the primary source of Nitrogen in plants and are required for protein and nucleic acid production (Durazzo et. al., 2013). Nevertheless, vegetables can accumulate high levels of nitrates and are thought to be the primary source of dietary nitrate intake. As a result, numerous reports on the possible adverse effects of humans exposed to nitrate on the ingestion of leaf vegetables have been conducted (Chung et al., 2004; Iammarino et al., 2013). This chemical compound is influenced by organisms, season, light, temperature, growth process, and fertilizer application. Nitrates are naturally found in leafy vegetables and certain root crops, with varying levels depending on the plant. Vegetables grown even though no nitrogen fertilizer is administered, high organic soils have a higher nitrate level than those fertilized soils. Guadagnin et al. (2005) also mentioned that hydroponic raised leafy vegetables have high amount of nitrate than those grown conventionally. In addition, Bryan and Loscalzo (2017) discussed that the content of nitrate in vegetables differ depending on the variety, development conditions, water supply, soil status, time of harvest, other factors specific for plants, nitrogen fertilization volume, type, timing, conditions of storage and processing methods. Even though vegetables contribute a big factor for nitrate consumption, Bryan and Loscalzo pointed out that the nitrate content of vegetables is a variable that can create a problem when evaluating nitrate's actual contribution to the nitrate/nitrite load in the diet. When the nitrate ion's absorption outnumbers its reduction and consequent absorption, vegetables have a tendency to accumulate nitrates. The structure of the soil wherein the plants are grown, the type of crop planted, the type of fertilization utilized, and the season of the year, as well as the ambient circumstances in which the crop grows, all contribute to this accumulation. Regardless, different plant types and cultivars have different quantities of nitrate. As a result, depending on the vegetables ingested, the consumer is susceptible to nitrates in varied ways. In addition, although vegetables are high in many critical elements that protect against chronic diseases (WHO, 2003), intensive farming may have higher concentration levels than in the past due to the higher use of commercial nitrogen fertilizer and animal manures. In regards to this, Tamme et. al., (2005) concluded in their studies that leafy vegetables can accumulate high amounts of nitrates and concentrations can reach up to 6,000 $mgkg^{-1}$. More so, Walters (1981) also come up with values of nitrates from their vegetables ranging largely between 1 and 1 $mg\ kg^{-1}$ of fresh samples and fruits range below 1 $mgkg^{-1}$ of fresh sample respectively.

Bryan (2017) reported several factors influence the nitrate concentration in plants, according to the study. It was also mentioned that as the daytime temperature drops below a suitable level, nitrate levels rise; therefore, geographical region and harvest season have an impact on nitrate content. Weightman et. al. (2006) further stated that in the same climate condition, winter sown crops have higher nitrate concentrations than the summer sown crops. Plants cultivated in the shaded places, at different altitudes with little sunlight, or during drought collect more nitrate than those growing in perfect water and light circumstances. In general, several factors can affect nitrate levels in vegetables by affecting one or more plant processes, such as nitrogen uptake, nitrogen transport, or nitrate reduction and assimilation (Bryan and Loscalzo, 2017). Nitrite concentrations in vegetables can increase as a result of bacterial contamination and endogenous catalase enzyme action, a greater proportion of the nitrate is converted to nitrite under undesirable post-harvest processing conditions; however, nitrite formation in vegetables is suppressed under chilled storage condition because the intrinsic nitrate enzymatic activity is neutralized. (European Food Safety Authority, 2008). The nitrite content of vegetables increases with nitrate concentration during storage at room temperature.

Human nitrate and nitrite toxicity varies greatly depending on dietary patterns, water source geography, and individual exposure to nitrogen oxides in the atmosphere. Vegetables accumulate high levels of nitrates and are believed to be the primary source of dietary nitrates. The link between nitrate and nitrite consumption and cancer, especially gastric and other gastrointestinal cancers, has piqued interest and has been thoroughly researched. As a result, numerous studies on the possible adverse effects of nitrate exposure on humans have been conducted on leaf vegetable consumption (Chung et al., 2004; Iammarino et al., 2013). The transformation to nitrite that produce methaemoglobin under the process of ingestion represents a significant hazard . If methaemoglobin would be unable to bind oxygen, the respiration curve shifts to the left, resulting in hypoxia. The derived nitrate has a high affinity for absorption by plants after oxidation by microorganisms. The consumption of nitrate and nitrite was investigated in the Diet, Physical Activity, and Cancer Prevention study, but the result was inadequate to make conclusions. Nonetheless, the study indicates that they may be carcinogens "under circumstances that encourage nitrosation" (WCRF/American Institute for Cancer Research, 2007). Several authors assumed that vegetable consumption accounts for the majority of total nitrate content in a typical diet. According to the European Union Food Commission (1992), the daily appropriate nitrate and nitrite intake is 0-3.65 mg and 0-0.07 mg, respectively. A 60 kg male's usual daily nitrate and nitrite consumption, according to the FAO and WHO food board, is 220 - 240 mg and 16 - 32 mg, respectively. As a result, the amount of certain foods contains high levels of nitrate and nitrite are monitored by EU and many other countries around the world (Gorenjak and Cencic, 2013). Externally, nitrates are consumed mainly from drinking water and food, which are the primary sources of consumption; constitutively, nitrates are a component of the nitrate-nitrite-nitric oxide route and are linked to important physiological features; exogenously, nitrates are easily absorbed mainly from water and food, which are the primary sources of ingestion. The biggest possibility for nitrate development is found in plant-based diets, particularly leafy and root vegetables such as spinach, lettuce, celery, and beets. (U.S. National Academy of Sciences, 1978; Petersen and Stoltze, 1999).

1.2 OBJECTIVES

The principal goal of this research was to determine the nitrate content of vegetables available in Taiwan during the summer and winter seasons, as well as to estimate the daily nitrate intake of Taiwanese people from leaf vegetable consumption. The impact of harvesting season (summer vs winter) was also investigated using the collected data. The possible health risks of nitrate exposure were then characterized using a probabilistic risk assessment approach.

CHAPTER 2
REVIEW OF RELATED LITERATURE

2.1 CHARACTERISTICS OF NITRATE

Nitrate is a molecular ion with a chemical formula of NO3-. This chemical compound consists of one nitrogen atom and three oxygen atoms in a trigonal planar arrangement. The ion of nitrate is not considered to be toxic but the reduction of nitrate by ingestion convert it to its toxic nitrite form. In agriculture, Nitrate is largely known for its purpose as a fertilizer in growing plants (Menard et. al., 2008). According to several studies, it was mentioned that the presence of this chemical compound is present most especially for vegetable and water. For this reason, end consumers are likely to be more exposed to these compounds even though it was discussed that the presence of nitrate is produced naturally. Nitrates originally found as a compound for nitrogen and has an ample amount that is present in soil, water and vegetables (Durazzo, et. al., 2013). This is also the reason why nitrate plays an important role in the food chain for every living organism. It is safe to consider that nitrates don't pose a threat to human health at a minimal concentration but the further metabolism and conversion to nitrite can associate the negative effect of nitrate to health due to the potential risk of gastrointestinal cancer and methaemoglobinaemia (Bryan et. al., 2017).

2.1.1 Nitrate in vegetables

The presence of nitrates in different agricultural product plays an important role to determine the quality and its influence to human health and most vegetables can store up a large amounts of nitrate. Nitrate itself is considered to be the major source of nitrogen (N) that it presents in crops. Whether as an extrinsic fertilizer or as an intrinsic ion, nitrate activates a collection of transcriptional regulators that shape shoot and root structure, affect blooming time, and alleviate spore germination. The concentration of nitrate present in vegetables may vary from the different factors like the variety, characteristics of the soil, influence of harvesting time, light, temperature and different ways of growing and application of fertilizers. According to Santamaria in 2006, nitrate-accumulating vegetables belong to the families *Brassicaceae* (rocket, radish, mustard), *Chenopodiaceae* (beetroot, Swiss chard, spinach), and *Amarantaceae;* however, species with high nitrate contents also belong to the *Asteraceae* (lettuce), and *Apipaceae* (celery, parsley). Santamaria went on to discuss that the differential capacities of vegetable species to collect nitrate could be due to the different sites of catalase enzyme activity, as well as the different degrees of nitrate accumulation and translocation in plant (Santamaria, 2006).

Table 1. Classification of vegetables according to nitrate content

Nitrate content (mg/100 g fresh weight)	Vegetable varieties
Very low, <200	Artichoke, Asparagus, Broad bean, Eggplant, Garlic, Onion, Green bean, Mushroom, Pea, Pepper, Potato, Summer squash, sweet potato, tomato, watermelon
Low, 200 to <500	Broccoli, Carrot, Cauliflower, Cucumber, Pumpkin, Chicory
Middle, 500 to <1000	Cabbage, Dill, Turnip, Savoy cabbage
Hiigh, 1000 to <2500	Celeriac, Chinese cabbage, Endive, Fennel, Kohlrabi, Leek, Parsley
Very high, >2500	Celery, Cress, Chervil, Lettuce, Red beetroot, Spinach, Rocket (rucola)

(derived from Santamaria, 2006)

2.1.2 Seasonal difference and storage and processing

During an untimely post-harvest storage situation, the nitrite concentration may rise in vegetable since that a big portion of nitrate is changed to nitrite due to the contamination of bacteria and its internal nitrate reduction. In addition, a consequential effect may happen in the reduction of nitrate and nitrite in vegetable mainly because of temperature and more importantly at its storage condition. On the other hand, the European Food Safety Authority (2008) mentioned that the process of pureeing is under the suspicion of releasing the internal nitrate reduction that cause too much reduction for nitrite most especially in vegetables. In addition, several studies had shown that the increase of nitrite level was discovered in home-made purees of vegetable after its refrigeration for twelve hours or more. Due to this observation, it has come to a conclusion that proper preparation and storage may contribute to a safe consumption of vegetables with high nitrate content (Chung et. al., 2003).

To the contrary, the European Union discussed that there is a distinct limit between the established concentration for spinach and lettuce that resulted from their cultivation in different season. High amount of nitrate is present for vegetables that were sown during winter time than those vegetables sown in summer (European Commission, 2001). However, Escobar- Gutierrez dispute those findings in year 2002 stating that as a result of their research; the concentration of nitrate emphasized a high availability between varieties and also for varieties with one cultivar showing that high concentration was much present during summer than in winter season. It simply indicates that according to their study, the concentration of nitrate has a big range of potential between different cultivars and the exceeding limit of concentration was more common during summer.

2.1.3 Human exposure to Nitrate

The exposure of nitric oxide and nitrogen dioxide through the atmosphere and environment, nitric oxide intrinsic production, oral exposure of nitrate by food, swallowing of saliva and including the drinking water were mainly the sources of nitrite and nitrate exposure (Bryan et. al., 2017). Among those factors mentioned, the exposure of humans to nitrates is primarily derived from its oral exposure through the dietary intake of vegetables. The reduction by oral consumption of nitrate has been largely known to contribute to the exposure of nitrite in humans (Eisenbrand et. al., 1980).

Nitrate itself contains a small amount of toxicity but it can still be transformed into nitrite that can cause the carcinogenic nitrosamine formation and methaemoglobinaemia. In addition to that, Bryan et. al. (2017) discussed and concluded several major criteria to be met in order to assess the possible exposure of humans towards nitrite and nitrate.

Hence, these are the following:

- First, the requirement for an appropriate database of the chemical constituents' sources in food, water, and any other relating source of exposure.

- Second, the requirement for precise criteria for estimating cumulative exposure to beverages that contain components to nitrosamine formation.
- Third, knowledge concerning the exposure from derived nitrosamine carcinogens since that nitrates and nitrites are not dangerous at normal exposure.
- Lastly, the requirement upon learning the amount of intrinsic and extrinsic nitrates and nitrites that are transformed to carcinogenic nitrosamines after consumption.

2.2 RISK ASSESSMENT THEORIES AND METHODOLOGIES

2.2.1 Risk

Risk by definition is described by Kammen and Hassenzahl (1962) as the probability that an outcome will occur times the consequence, or level of impact, should that outcome occur. Understanding different values and having an in depth description can help to identify possible outcome of risk and its probability to make better decisions. Much rather, risk analysis answers to the demand of interested groups and individuals affected. In addition to that, risk analysis' main intention is to inform and not to provide and set certain decisions. In the past years, risk analysis had made a great impact for legislation not only from their locality but to the international level as well (Kammen et. al., 1962). Based on the book written by Robson and Toscano (2007), risks' main purpose is to define its function for hazard and exposure. Perceptions of risk is necessary especially for public to help them have the knowledge about different policies in regards to risk. Moreover, risk analysis had gained interest by some government officials in some countries as a great tool in implementing certain environmental decisions. During the year 1985, Covello and Mumpower also had mentioned that risk analysis had been widely used for over a period of years in one form or another.

2.2.2 Risk Assessment

Risk assessment is a systematic application of regulations and experimental methods to characterize the hazard related to human exposure on various substances. The acquired information provided by this assessment can then be applied for the regulation of using chemical substance and even so, for its political, social, economic, and technical deliberation under the process of managing the risk (Nielsen et. al., 2008). In addition, risk assessment is the process of identifying a hazard and attempting to limit or evaluate its level of potential harm under a specific set of conditions. The possible scenarios, it's likeliness to happen or probability to happen, and the possible consequences describing its adverse effects are the main reason why most of health enthusiast are eager to assess different causes regarding to risk assessment. In addition to that, Robson and Toscano (2007) mentioned in their book that balancing the benefit and risk, setting the target level of risk, setting of priorities for different program activities, and estimating the residual risk and its extent at reduction after certain steps are done for reducing the risk also are describe to be common objective for risk assessment.

In addressing exposure's risk towards health against hazards present in the environment, there are three interrelated processes that mainly focus in making decisions and actions in relation to environment's risk. First one is the assessment of risk that defines the process of analyzation and characterization of different information about risk. Next one is the managing of risk wherein the process for the integration of the results is done in relation to decision making together with its social, economic, political, regulatory and even other information related in order to manage the risk. Third is the risk communication wherein discussion with stakeholders happen to determine information that may improve risk assessment and to inform stakeholders about the possible consequences of risk management decisions. After thorough gathering and analyzing all the information gathered, a risk policy is then established to provide guidance in managing and mitigating possible negative outcomes that may take effect (Nelson et. al, 2007).

2.2.3 Organizations involved in Risk Assessment of Chemical Substances

Nielsen et. al. (2008) mentioned that there are different organizations that specializes in supporting the studies and other chemical risk assessment activities. United Nations (UN) as an example is the first and the superior among other international organization to participate in chemical risk assessment. Having UN as the spear head for this organization, other specialized agencies were also established to set different objectives and classifications involved in risk assessment in regards to different chemical substances. They are the following:

- World Health Organization (WHO)
- Organization for Economic Co – operation and Development (OECD)
- United States of America (US federal bodies)
- The European Union (EU)
- Globally Harmonized System of Classification and Labeling of Chemicals (GHS)

(derived from Nielsen et. al., 2008)

In every specialized agency that were gathered and collected by Nielsen et. al. (2008) there were different organizations that was established to provide different set of objectives, values and legislations that are essential in providing information for different chemical substances in relation to their risk assessment. (see Figure 2 at the next page)

- International Programme on Chemical Safety **(IPCS)**
- Food and Agriculture Organization of United Nations **(FAO)**
- Joint FAO/WHO Expert Committee on Food Additives **(JECFA)**
- Joint FAO/WHO Meeting on Pesticides Residues **(JMPR)**
- Inter-Organization Programme for the Sound Management of Chemicals **(IOMC)**
- International Agency for Research on Cancer **(IARC)**
- **IPCS:** Chemicals Assessment

- The **OECD** Chemical Programs
- Mutual Acceptance of Data **(MAD)**
- The **OECD** Existing Chemicals Program
- The **OECD** New Chemicals Program
- The **OECD** Pesticides and Biocides Program
- **OECD** Test Guidelines

- United States Environmental Protection Agency **(US-EPA)**
- Agency for Toxic Substances and Disease Registry **(ATSDR)**
- National Toxicology Program **(NTP)**
- United States Food and Drug Administration **(US-FDA)**

- EU Chemical Programs
- Harmonization of Risk Assessment in DG SANCO

Figure 1 : Superior Organization under its specialized agency together with their corresponding different international bodies

2.2.4 Human Health Risk Exposure and Assessment

Dating back during 1980’s, most of the risk assessments such as the health, environmental and even technological have been keen to depend on the use of the basic paradigm for human health risk assessment that was established by the National Academy of Sciences’ National Research Council (National Research Council, 1983). This paradigm evaluates a four-step procedure in analyzing the data to summarize different implications that can be followed and understand. For every steps being described on the paradigm, relevant and scientific based reliable information were also evaluated. The four steps in the paradigm that was mentioned by NRC are the: Hazard identification, Dose- response assessment, Exposure assessment and lastly, the Risk characterization. In addition, broadening the application beyond the health risk assessment in order to formulate the problem is somewhat evolved and recognized as a first step for every risk assessment procedure. By planning and scoping, problem formulation supplies the opportunity for the risk assessors to properly define what could be the problem that is being addressed (Robson et. al., 2007). Furthermore, Robson and Toscano (2007) further evaluate every description and characterization of the steps being described by the paradigm provided by the NCR.

- ❖ **Hazard Identification** – determine the exposure potential of an agent to cause a high incident of undesirable effect for the environment or human health. In depth understanding of mode-of-action (MOD) is very necessary for this step. This understanding help to supply the basis of application of results between its toxicological properties to better characterize the hazard.
- ❖ **Dose-Response Assessment** – this assessment is considered to be attempts that place the quantitative measures on the magnitude of hazards in questions. In addition, it is also the making of a quantitative evaluation in regards to the incident of any adverse effect that is expected as a result of exposure to a particular amount of a contaminant.
- ❖ **Exposure Assessment** – this assessment determines the process on how extent it is for humans, animals, or other life forms are exposed to any hazardous substance. Certain exposures can also be measured by their concentrations or even its duration and frequency of the substance that is present in the environment. In addition, exposure assessment describes the target population, exposure pathway such as

inhalation, ingestion and dermal absorption, lastly this assessment defines the potential hazard a certain substance can attain through estimating its cancer and non-cancer risk potential.

- **Risk Characterization** - it is the definition between the risks' nature and magnitude to health, environment, other life forms and also, its attendant to uncertainties. Risk characterization is the result from the combination of results obtain from the analysis of effects (dose-response) and exposure assessment.

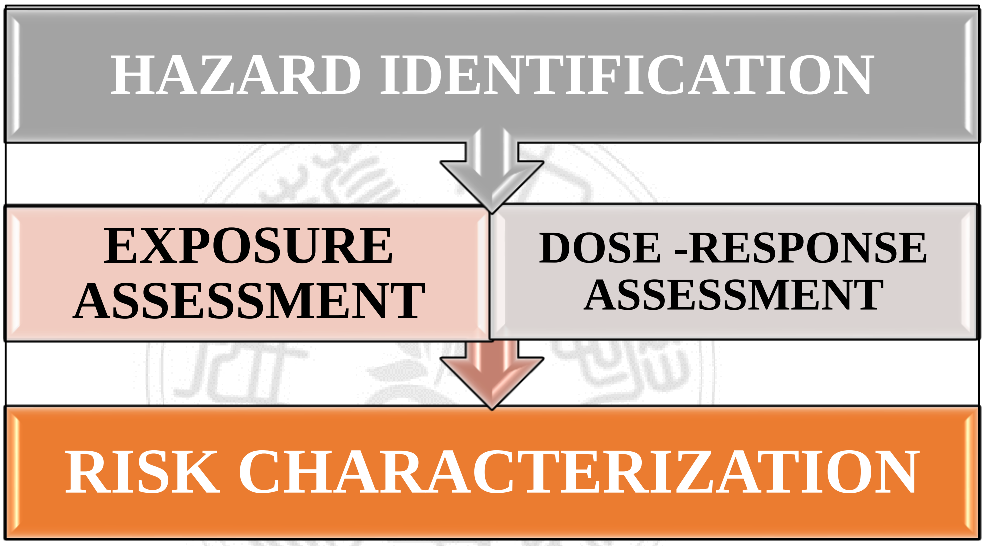

Figure 2: Risk Assessment Paradigm (U.S. EPA, 2002)

CHAPTER 3
MATERIALS AND METHODS

3.1 SAMPLE COLLECTION

Carrying out risk assessment on the dietary intake of vegetables was done through a wide monitoring program in Taiwan during the year 2011 and 2012. A total of 48 vegetables and 404 total samples were collected and analyze through Ion Chromatographic Analysis done at the Post-Modern Agriculture department laboratory at Mingdao University. The nitrate content of vegetables under the influence of harvesting time (winter and summer) are selected and separated from these raw data provided. A total of 30 vegetables with 83 overall samples and 30 vegetables with 103 overall samples during summer and winter respectively was collected and separated by the initial raw data provided.

Table 2. Vegetable samples used during summer and their corresponding range, mean and standard deviation values

NAME OF VEGETABLE	RANGE	MEAN	STANDARD DEVIATION
LETTUCE	385- 3010	1187.167	943.1007
SPINACH	1392-4490	2522.75	1389.566
AMARANTH	2609-5715	4387	1164.016
CHINESE CABBAGE	884- 4236	1977.5	1407.667
WATER SPINACH	1520-3053	2244.286	529.853
SWEET POTATO LEAVES	654- 5097	1948.444	1813.987
HYDROPONIC CABBAGE	4852-9193	6848.333	2191.363
ORGANIC PECHAY	2573-7321	4947	3357.343
PECHAY	2208-2800	2504	418.6072
ROMAINE "COASTAL STAR" LETTUCE	317-985	554	373.8756
ORGANIC PAK - CHOI	5256-5384	5320	90.50967
PAK-CHOI	6467-6997	6732	374.7666

ORGANIC GREEN MUSTARD	3850-6750	5388	1457.989
ORGANIC AMARANTH	5900-6600	6250	494.9747
MALABAR SPINACH	985- 1217	1127.333	124.6448
RAPE	2808*	2808	
CHINESE CABBAGE (spp)	5020*	5020	
YELLOW ZUCCHINI	856- 980	918	87.68124
GREEN ZUCCHINI	850- 877	863.5	19.09188
CARROT	250*	250	
WHITE RADISH	740*	740	
CABBAGE TURNIP	330*	330	
CELERY	302*	302	
ORGANIC CANOLA	1500*	1500	
ORGANIC SPINACH	100*	100	
GREEN PEPPER	25- 88	56.5	44.54773
RADISH	980*	980	
TOMATO	44- 80	62	25.45584
COW TOMATO	35- 120	77.5	60.10408
ORGANIC PAK-CHOI (spp)	862*	862	

* one sample available

Table 3. Vegetable samples used during winter and their corresponding range, mean and standard deviation values

NAME OF VEGETABLE	RANGE	MEAN	STANDARD DEVIATION
LETTUCE	177-1954	756.8889	636.6664
SPINACH	1311-4732	3155.923	1104.479
AMARANTH	2198-5502	4229	1777.646
CHINESE CABBAGE	77-1041	714	551.7237
WATER SPINACH	26-3996	2288.8	1939.169
SWEET POTATO LEAVES	1054-1368	1211	222.0315
HYDROPONIC CABBAGE	5843-6058	5950.5	152.028
PECHAY	370-4497	2197.692	1281.449
ROMAINE “COASTAL STAR” LETTUCE	313-2760	1225.714	766.0417
BOK-CHOI	5855-895	3126.2	2112.345
CABBAGE	272-395	347.6667	66.2143

ROMAINE LETTUCE	2589-2333	2053.5	322.4257
KALE	1194-2940	2067	1234.608
CHRYSANTHEMUM LEAVES	2706*	2706	
RAPE	2754-3998	3233	669.5006
CHINESE CABBAGE (spp)	2617-5184	4295.4	1072.909
YELLOW ZUCCHINI	1052*	1052	
GREEN ZUCCHINI	1073*	1073	
SMALL CHINESE CABBAGE	2887*	2887	
NAPA CABBAGE	770*	770	
CHRYSANTHEMUM LEAVES (spp)	622-914	768	206.4752
BROCCOLI	26- 188	114	81.90238
BROCCOLI (spp)	55- 85	68	15.3948
WHITE RADISH	572*	572	
ROMAINE LETTUCE (spp)	219- 233	226	9.899495
CABBAGE TURNIP	384- 483	433.5	70.00357
CELERY	463*	463	
FENNEL	1749*	1749	
TOMATO	49-55	52	4.242641
COW TOMATO	28-32	30	2.828427

* one sample available

3.2 EXPOSURE ASSESSMENT FOR NITRATE

Exposure to different chemical substance varies to different routes of exposure. In this study, the exposure of nitrate was by consumption through the ingestion of vegetables. Vegetables can be considered as staple food like rice in Taiwan since it also has a large market value for the consumption of the residents in the country. Exposure of these vegetables therefore can raise a concern in the health of the population.

The estimated daily intake (DI) therefore of nitrate consumption can be calculated as:

$$DI = (Cveg \times IR \times CF) / BW \quad \text{(Eq. 1)}$$

Wherein the total daily intake (DI) was calculated from the values derived from the total concentration of vegetables (Cveg), ingestion rate (IR) value derived from the National Feeding Database, body weight (BW) from the average values for both male and female adults and lastly, a conversion factor (CF) from kilogram to gram was also derived to balance the given equation.

In this study, the parameters were separated in this equation to compare different values between male and female adult respondents aged 19 to 44 and also for the difference of the values during summer and winter.

Table 4. Parameters use for DI calculation

PARAMETER (WINTER)	SYMBOL	UNIT	SOURCE
Vegetable Concentration	Cveg	mg / (kg*day)	This study
Ingestion Rate	IR	mg/day	National Feeding Database
Body Weight	BW	kg	潘文涵 (2016)
Conversion Factor	CF	kg/g	

3.3 HEALTH RISK ASSESSMENT FOR NITRATE

Based on the paradigm derived from the U.S. EPA (2002), the different stages mentioned are used in order to calculate the risk of nitrate exposure. The characterization for the risk is done in order to evaluate the exposure of dose derived from the given values from the route of exposure. Hence, the non-carcinogenic (HQ) and carcinogenic (TR) values were calculated in order to evaluate the risk impose from nitrate exposure.

$$HQ = DI / RfD \qquad (Eq.\ 2)$$

From the given equation, the reference dose has a given value of 1.6 mg/kg/day which was derived from the Integrated Risk Information System (IRIS) database. The USEPA's standard value for the health risk assessment is 1. Therefore, if the derived value was greater than 1, the non-cancer risk for the substance exceed its limit of acceptability, hence, if the value derived was lower than 1, the non-carcinogenic risk is still at its acceptable limit (USEPA, 2014).

Moreover, the equation for calculating the cancerous risk of nitrate is expressed as the target risk (TR) value with the equation shown as:

$$\mathbf{TR = DI \times SF} \quad \textbf{(Eq. 3)}$$

Wherein the TR value for nitrate in the given equation has a slope factor value of 10^{-5} (kg*day) /mg (Duvva et al., 2021). The acceptable TR value is $1\ x\ 10^{-6}$, if the values derived from the equation is lower than 10^{-6} , it can still be evaluate as *negligible* risk, for cancer risk between 10^{-6} and 10^{-4} it can be evaluate as an *acceptable* risk. Lastly, if values derived for cancer risk is higher than 10^{-4} it is evaluated as an *unacceptable* risk and considered as a potential risk for cancer.

Table 5. Reference Dose (Rfd) and Slope Factor (SF) values for HQ and TR

	VALUE	UNIT	SOURCE
REFERENCE DOSE (RfD)	1.6	mg/kg/day	IRIS Database
SLOPE FACTOR (SF)	10^{-5}	(kg*day) /mg	Duvva et al., 2021

3.4 STATISTICAL ANALYSIS

Monte Carlo simulations signify the probability of different outcomes in such a strategy that cannot simply be normal due to the involvement of unusual factors. It's a method that enables you to see the impact of risk and sensitivity through forecast and articulation models. Monte Carlo simulation performs risk analysis by developing models of possible outcomes by substituting a variety of characteristics for any issue with inherent vulnerability.

The Monte Carlo method was done through the use of Oracle Crystal Ball software version 11.1. In order to carry out the risk assessment and to gather its statistical data, the values are fit to their best distribution using the software. After the distribution, the forecast of each of each value was then define in order to identify its output variables. After the forecast definition, the given distribution was then calculated for their risk probability. A number of trials was set so that the best nearest value can be attained using the simulation. Using the software, 10,000 set of trials was used and the assessment for risk and its corresponding values were attained after the simulation process. Sensitivity values were also gathered including also the values from 2.5, 25, 50, 95, and 97.5 percentile corresponding values for the different parameters and also for the results for risk characterization.

CHAPTER 4
RESULTS AND DISCUSSION

4.1 NITRATE CONCENTRATION IN VEGETABLES

Based on the given data from Table 2 and 3, there were 186 total samples used taken from the initial data of 404 samples and was separated and pulled together to derive the sample value of 83 concentration data for summer and 103 concentration data for winter. These data were separated based on the months of June to August (summer) and December to February (winter).

After pulling all the data's together, the values were fit according to their most appropriate distribution. The total concentration of vegetables (Cveg) was fit as a lognormal (LN) distribution. Since that nitrate concentrations are large in their numerical values; geometric mean and geometric standard deviation was used to describe each data.

4.2 RISK ASSESSMENT AND ANALYSIS EVALUATION

4.2.1 Evaluation for Nitrate exposure

Ingestion rate (IR) values were derived from the National Feeding database. Values gathered were based from adult male and female between the age of 19-44. From the normal distribution of IR data; a mean value of 303.76 and 309.12 and standard deviation of 250.33 and 245.49 was derived for male and female respondents respectively. Since that using of the normal distribution for the standard value of IR is relatively high, the data was fit as a lognormal distribution to avoid the result of negative values during the assimilation process. Corresponding values were also expressed as geometric mean and geometric standard deviation. As further mentioned, body weight (BW) was derived from the age 19-44 (adult) male and female Taiwanese respondent and values were fit as normal distribution together with its corresponding mean and standard deviation. Values mentioned can also be seen from tables 3 and 4 illustrating the values for ingestion rate, and body weight for both summer and winter season.

Through these parameters gathered altogether the total DI values were calculated using the formula for DI (Eq. 1) wherein female respondents show the highest estimated DI values compared to male for both summer and winter season. In addition to that, summer season has the highest DI values as expressed at 97.5 percentile.

Table 6. Input values used for DI calculation during summer

PARAMETER (SUMMER)	SYMBOL	INPUT VALUE	UNIT	SOURCE
Vegetable Concentration	Cveg	Male: LN (1908.84 , 2.51)[a] Female: LN (1908.84 , 2.51)[a]	mg / (kg*day)	This study
Ingestion Rate	IR	Male: LN (9.81 , 1.22)[b] Female: LN (9.81 , 1.22)[b]	mg/day	National Feeding Database
Body Weight	BW	Male: N (72.30 , 18.16)[c] Female: N (58.40 ,17.16)[c]	kg	潘文涵 (2016)
Conversion Factor	CF	**0.001**	kg/g	

values were shown as: (geometric mean ,geometric standard deviation)[a,b] ; (mean ,standard deviation)[c]

Table 7. Input values used for DI calculation during winter

PARAMETER (WINTER)	SYMBOL	INPUT VALUE	UNIT	SOURCE
Vegetable Concentration	Cveg	Male: LN (1119.14 , 3.48)[a] Female: LN (1119.14 , 3.48)[a]	mg / (kg*day)	This study
Ingestion Rate	IR	Male: LN (9.81 , 1.22)[b] Female: LN (9.81 , 1.22)[b]	mg/day	National Feeding Database
Body Weight	BW	Male: N (72.30 , 18.16)[c] Female: N (58.40 , 17.16)[c]	kg	潘文涵 (2016)
Conversion Factor	CF	0.001	kg/g	

values were shown as: (geometric mean ,geometric standard deviation)[a,b] ; (mean ,standard deviation)[c]

Table 8. DI values for both male and female respondents during summer and winter

Percentiles	2.5%	25%	50%	95%	97.5%
male	0.007 , 0.003	0.10 , 0.08	0.23 , 0.18	1.32 , 1.09	1.83 , 1.45
female	0.007 , 0.002	0.13 , 0.09	0.28 , 0.22	1.73 , 1.45	2.40 , 2.01

Percentile values are shown as (summer , winter)

Through the values from the sensitivity data (Figure 4 and 5), Cveg values has the great impact on the result from the DI values for both male and female during summer and winter season.

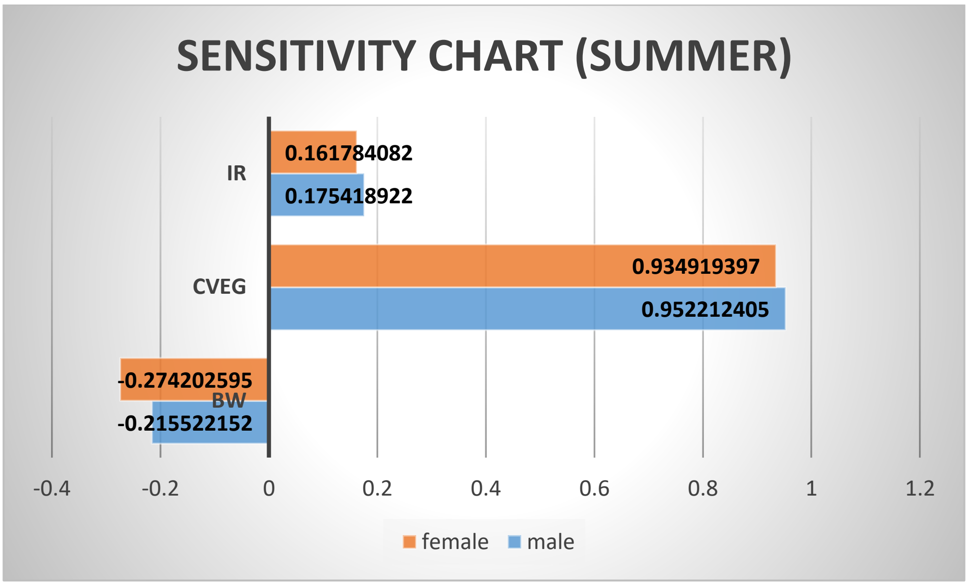

Figure 3: Sensitivity chart and values for summer

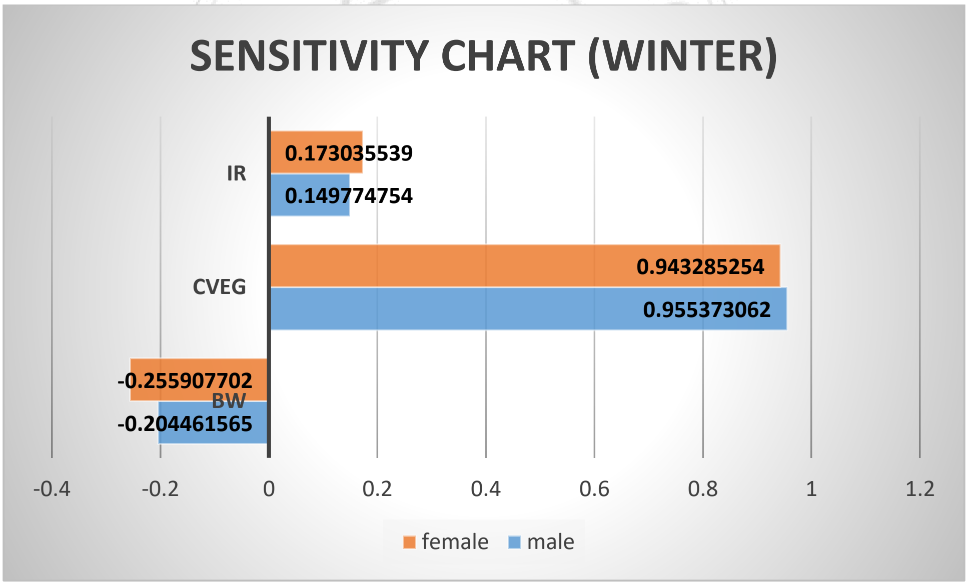

Figure 4: Sensitivity chart and values for winter

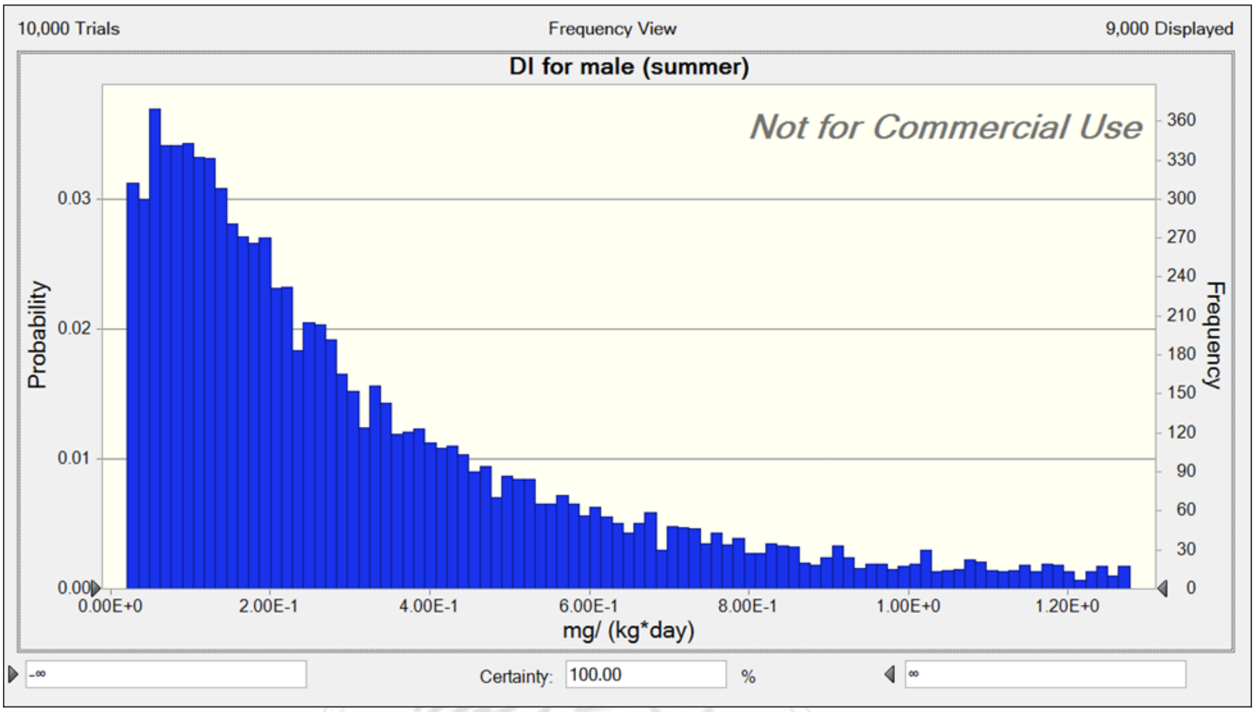

Figure 5: Daily intake graph for male (summer)

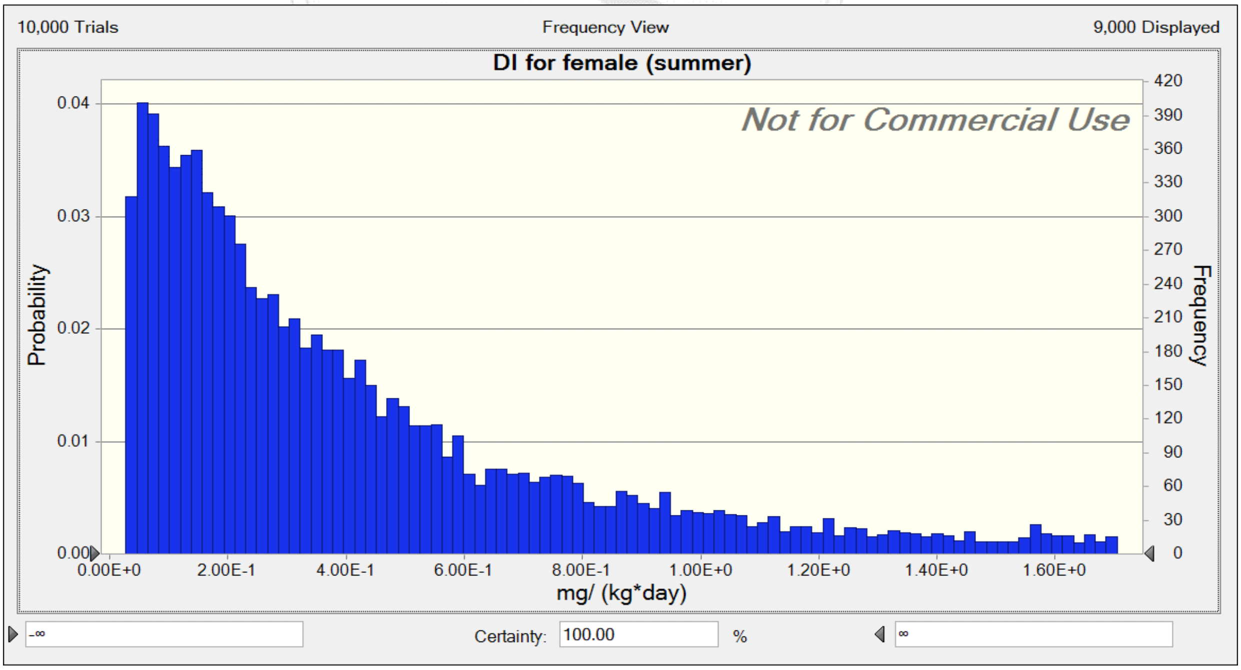

Figure 6: Daily intake graph for female (summer)

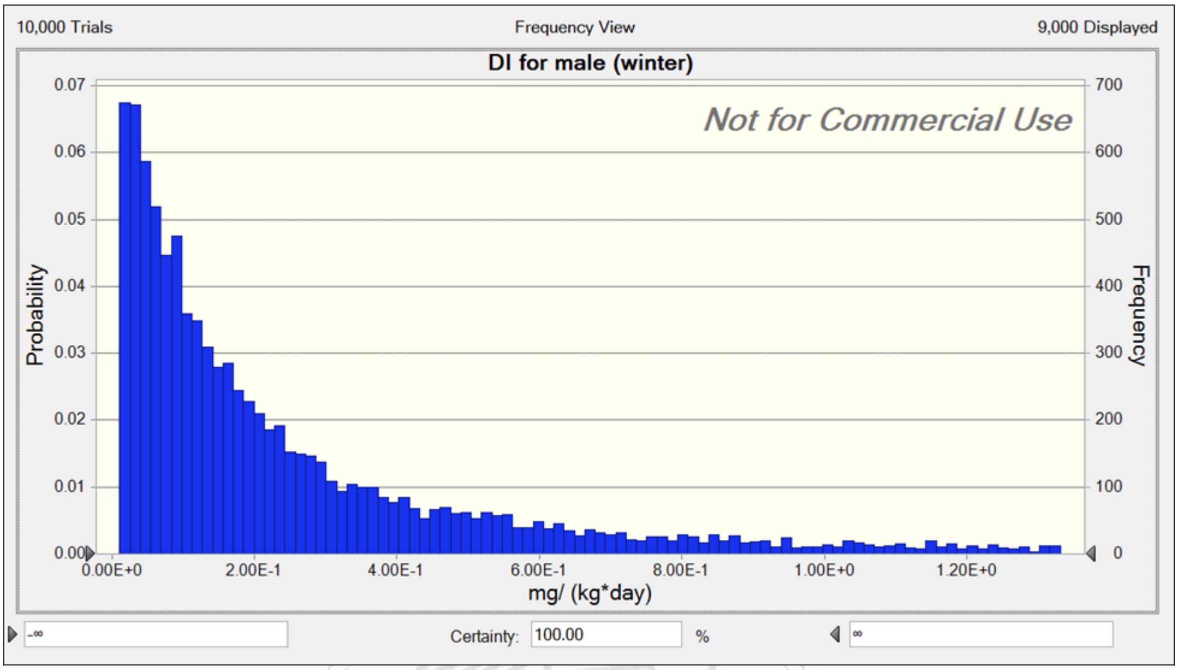

Figure 7: Daily intake graph for male (winter)

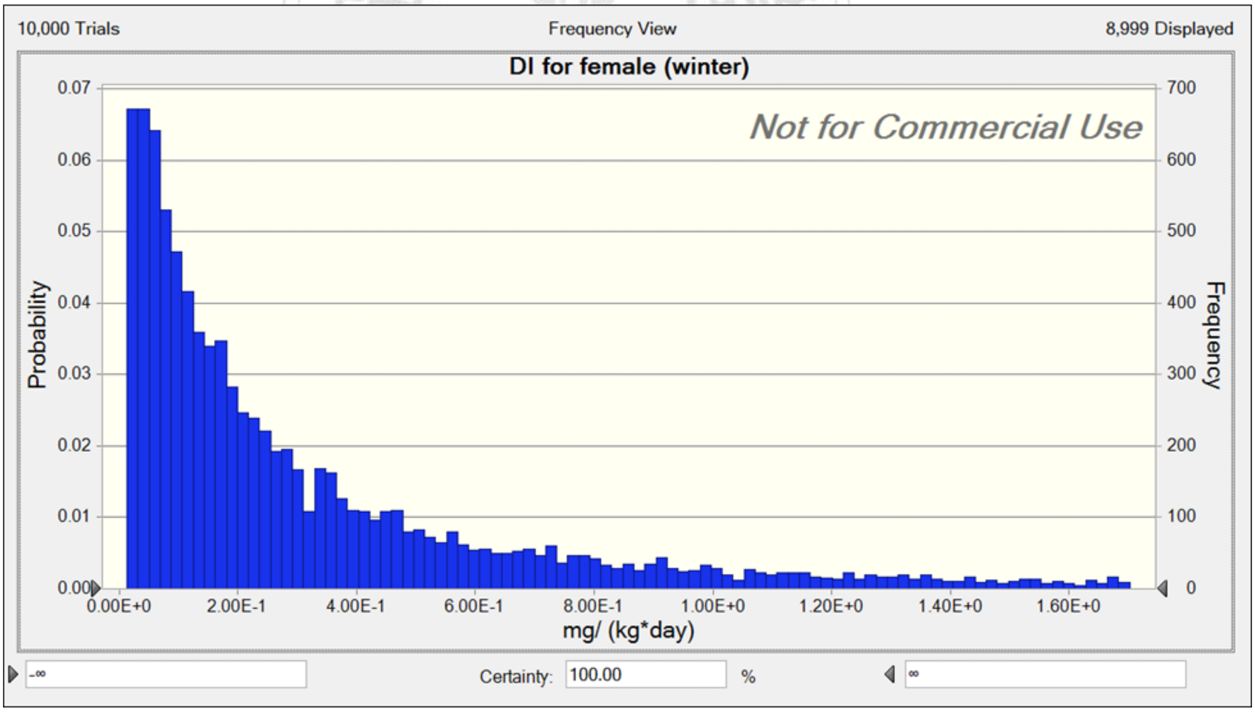

Figure 8: Daily intake graph for female (winter)

4.2.2 Risk Characterization

4.2.2.1 Non – Carcinogenic Risk

Based on the data provided from the IRIS database, nitrate is considered to be non-carcinogenic. However, toxicity of this substance can still raise concern because the reduction of nitrate to its nitrite form may cause gastrointestinal cancer and methaemoglobinaemia that may impose harm to human health. In this study, the non-carcinogenic risk was expressed through calculating the values derived from HQ equation (Eq. 2)

From the given equation, the reference dose has a given value of 1.6 mg/kg/day which was derived from the Integrated Risk Information System (IRIS) database. The USEPA's standard value for the health risk assessment is 1. Therefore, if the derived value was greater than 1, the non-carcinogenic risk for the substance exceed its limit of acceptability, hence, if the value derived was lower than 1, the non-carcinogenic risk is still at its acceptable limit (USEPA, 2014).

As a result, the mean value from the HQ computation shows a lower value from the standard value provided by the USEPA which means that during the average case scenario, HQ is still at its acceptable limit. Moreover, the predicted upper 95% confidence limit of *HQs* were lower than 1 except for female during summer, indicating the potential non-cancer risk of nitrate associated with vegetable consumption for Taiwan residents was acceptable and female respondents during summer should be take noticed. .

Table 9. Mean and 95% upper confidence limit HQ values and their corresponding meaning

	MEANING	VALUE
MEAN	average case scenario	Male: 0.24 , 0.20 Female: 0.32 , 0.26
Reasonable Maximum Exposure, **RME** (95% upper confidence limit of the mean)	worst case scenario	Male: 0.83 , 0.68 Female: 1.08 , 0.91

values are shown as (summer , winter)

In addition, values derived from mean and 95% upper confidence limit (as shown in Table 9) illustrate the difference of values between season and gender. For instance, for the mean value during summer and winter, higher value was derived from summer for both male and female respondents and the winter value was lower. On the other hand, during the worst case scenario for the hazard quotient, only female respondents during summer time is greater than 1 which means that the potencial non cancer risk for female during summer time is unacceptable and also , similar to the mean value, values derived at the upper confidence limit shows that summer still has a high value than winter and female respondents were still having the highest value of exposure.

Table 10. HQ values for both male and female respondents during summer and winter

Percentiles	2.5%	25%	50%	95%	97.5%
male	0.005 , 0.003	0.06 , 0.05	0.14 , 0.18	0.83 , 0.68	1.14 , 0.91
female	0.005 , 0.003	0.08 , 0.06	0.18 , 0.14	1.08 , 0.91	1.50 , 1.26

Percentile values are shown as (summer , winter)

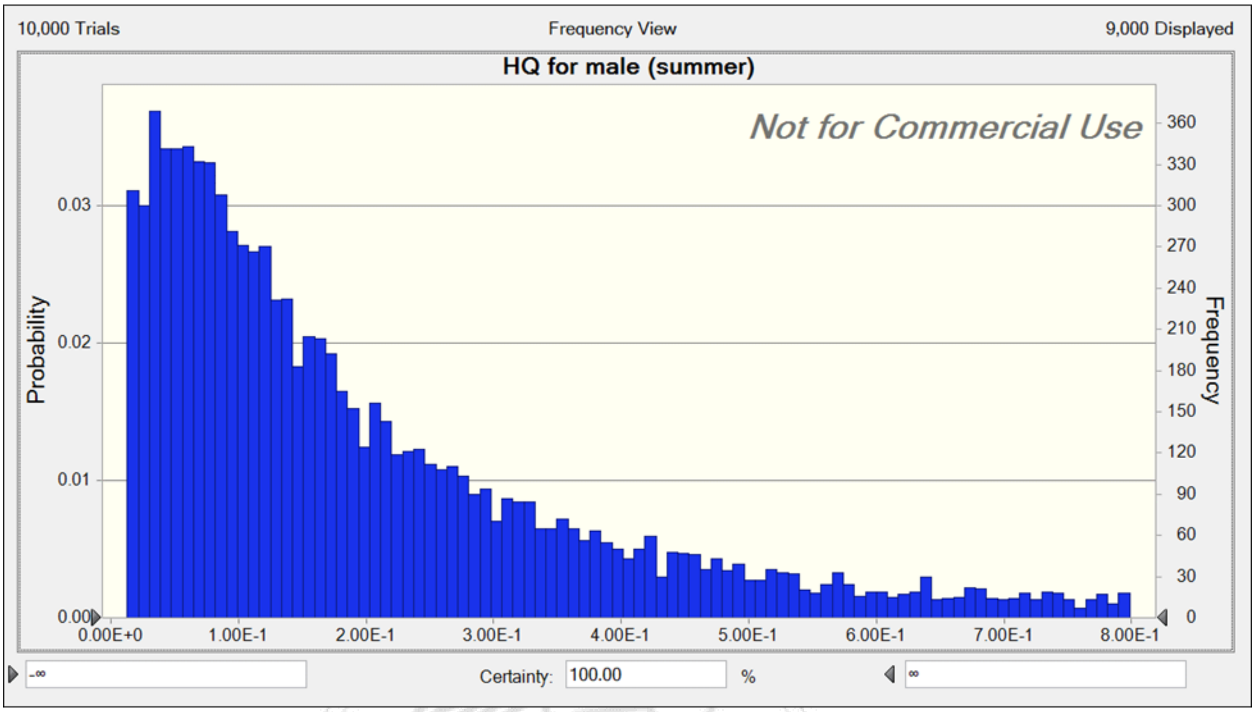

Figure 9: Hazard Quotient graph for male (summer)

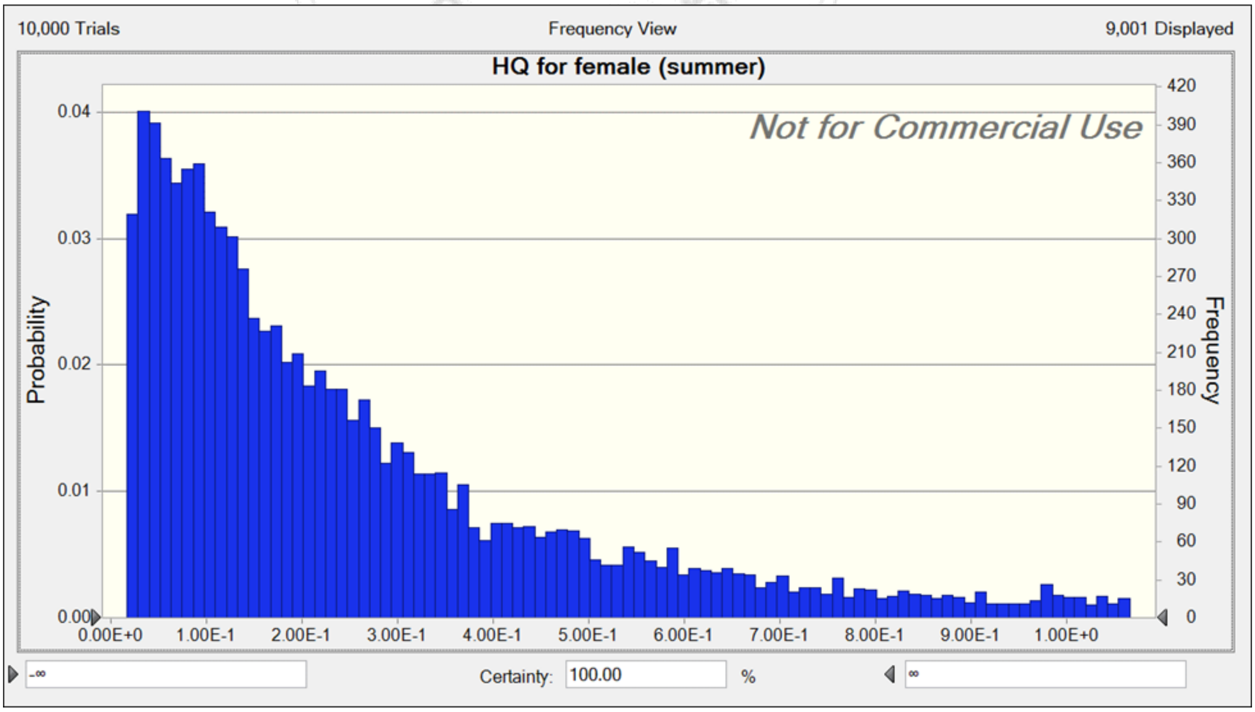

Figure 10: Hazard Quotient graph for female (summer)

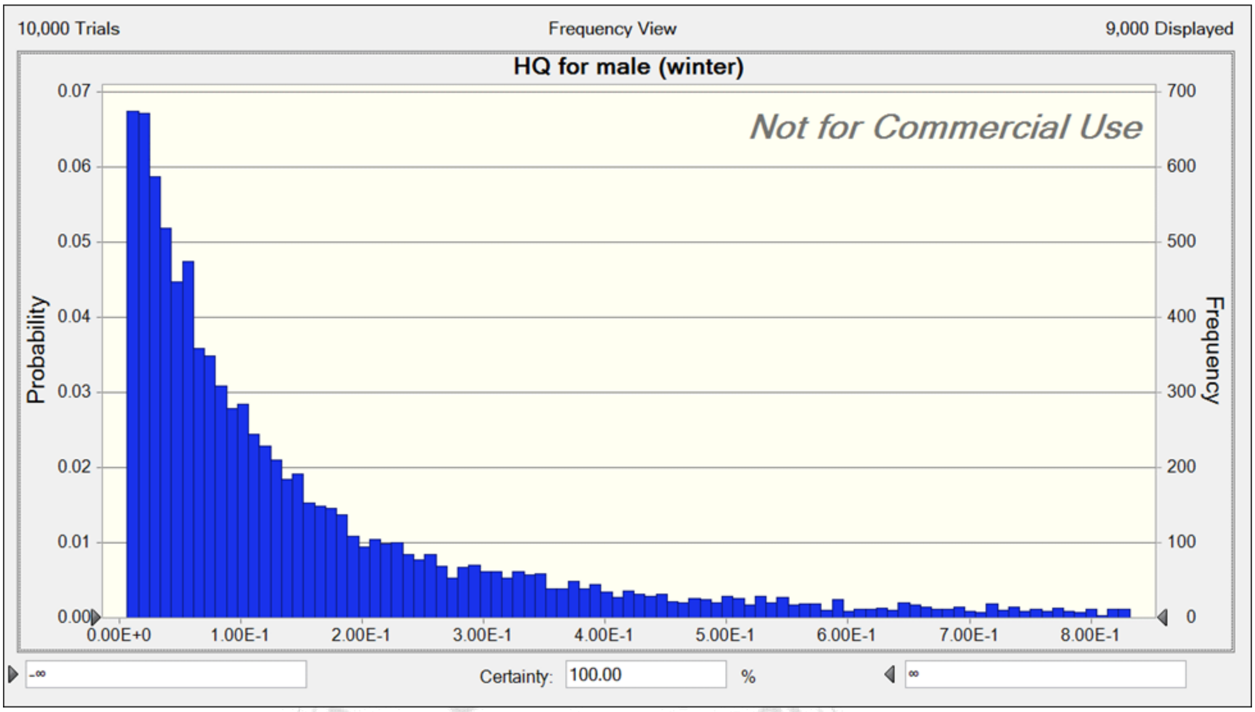

Figure 11: Hazard Quotient chart for male (winter)

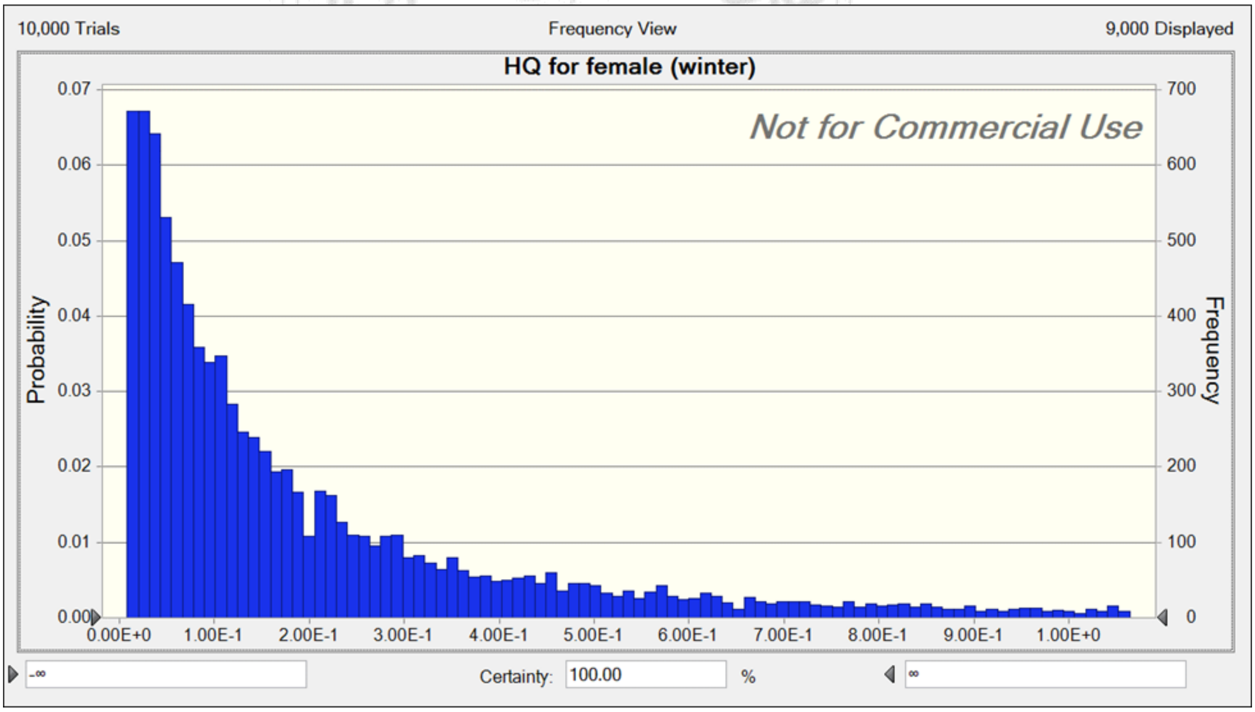

Figure 12: Hazard Quotient chart for female (winter)

4.2.2.2 Carcinogenic Risk

Carcinogenic risk describes the cancerous risk of a certain substance. In this study, nitrate concentration was already described as non-carcinogenic based on the IRIS database. However, carcinogenic risk was still computed on this data to show if there is any probability that certain risk may happen if data are derived from different seasons.

Moreover, as mentioned from Chapter 3 of this paper, the equation for calculating the cancerous risk of nitrate is expressed as the result from TR using the equation 3.

Wherein the TR value for nitrate in the given equation has a slope factor value of 10^{-5} (kg*day) /mg (Duvva et al., 2021). The acceptable TR value is $1x10^{-6}$, if the values derived for the cancer risk is lower than 10^{-6} it is defined as a “negligible” risk. Moreover, cancer risk values between 10^{-6} and 10^{-4} are expressed as an “acceptable” risk. Lastly, for cancer risk higher than 10^{-4} it is defined as an “unacceptable” risk.

Table 11. Mean and 95% upper confidence limit TR values and their corresponding meaning

	MEANING	VALUE
MEAN	average case scenario	Male: 3.98×10^{-6} , 3.20×10^{-7} Female: 5.17×10^{-6} , 4.19×10^{-6}
Reasonable Maximum Exposure, **RME** (95% upper confidence limit of the mean	worst case scenario	Male: 1.33×10^{-5} , 1.09×10^{-5} Female: 1.73×10^{-5} , 1.45×10^{-5}

Percentile values are shown as (summer , winter)

As a result, the upper 95% confidence limit of *TR* for male and female was respectively 1.33 x10^{-5} and 1.73 x10^{-5} during summer, and was 1.09 x10^{-5} and 1.45 x10^{-5} for winter time. This results showed that all cancer risk (*TR*) value ranged between the negligible level

(10^{-6}) and acceptable level (10^{-4}). Therefore, the cancer risk of nitrate associated with vegetables consumption should be noticed.

Table 12. TR values for both male and female respondents during summer and winter

Percentiles	2.5%	25%	50%	95%	97.5%
male	7.5×10^{-8}, 3.08×10^{-8}	1.02×10^{-6}, 7.73×10^{-7}	2.29×10^{-6}, 1.78×10^{-6}	1.33×10^{-5}, 1.09×10^{-5}	1.83×10^{-5}, 1.45×10^{-5}
female	7.66×10^{-8}, 2.98×10^{-8}	1.26×10^{-6}, 9.32×10^{-7}	2.83×10^{-6}, 2.19×10^{-6}	1.73×10^{-5}, 1.45×10^{-5}	2.41×10^{-5}, 2.01×10^{-5}

Percentile values are shown as (summer , winter)

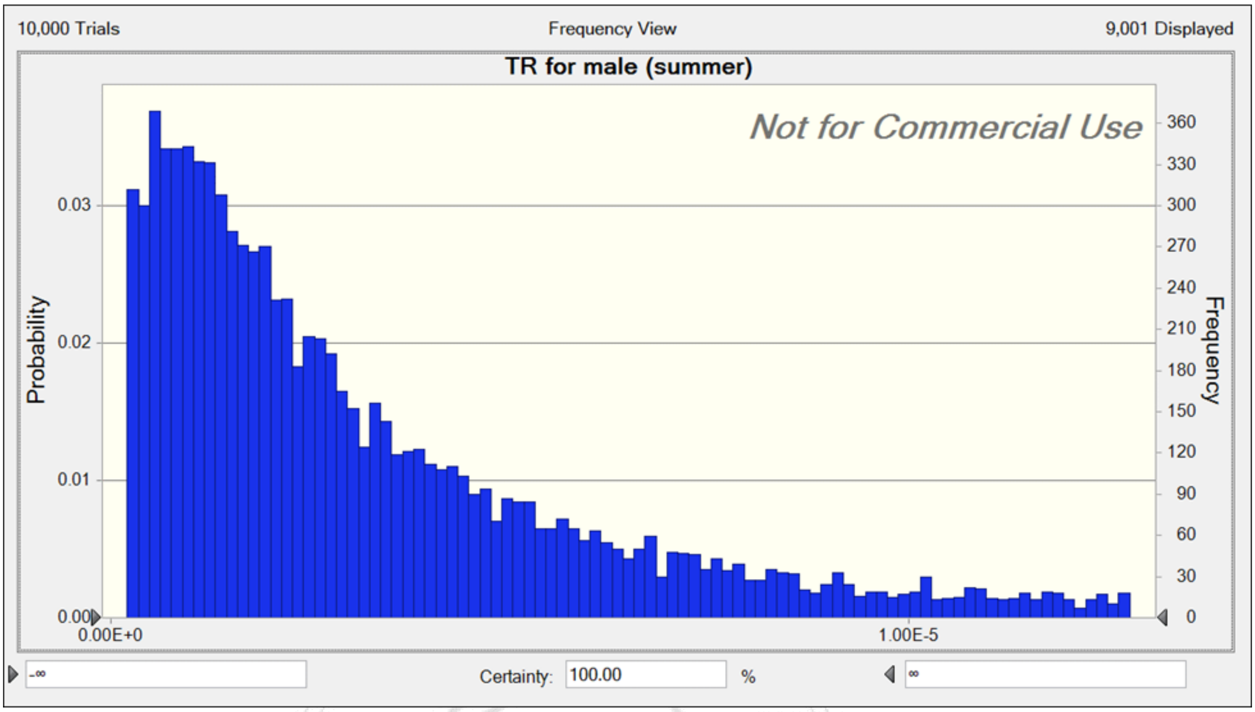

Figure 13 :Target Risk graph for male (summer)

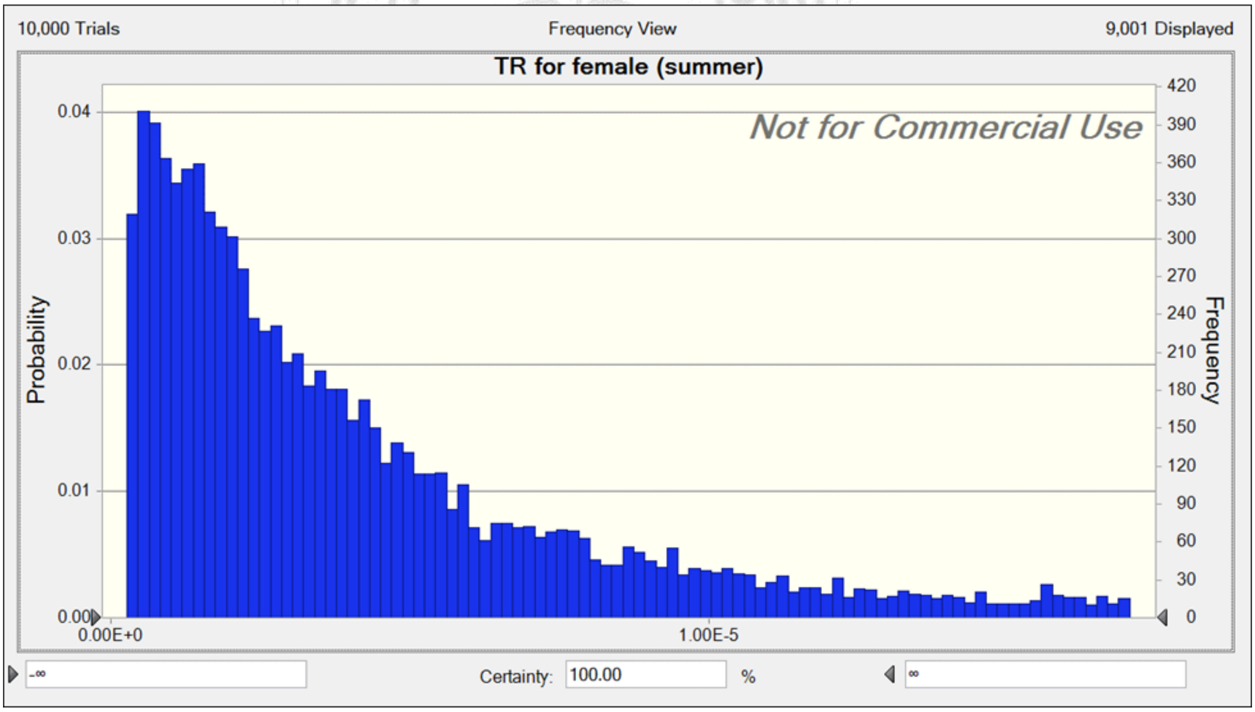

Figure 14 : Target Risk graph for female (summer)

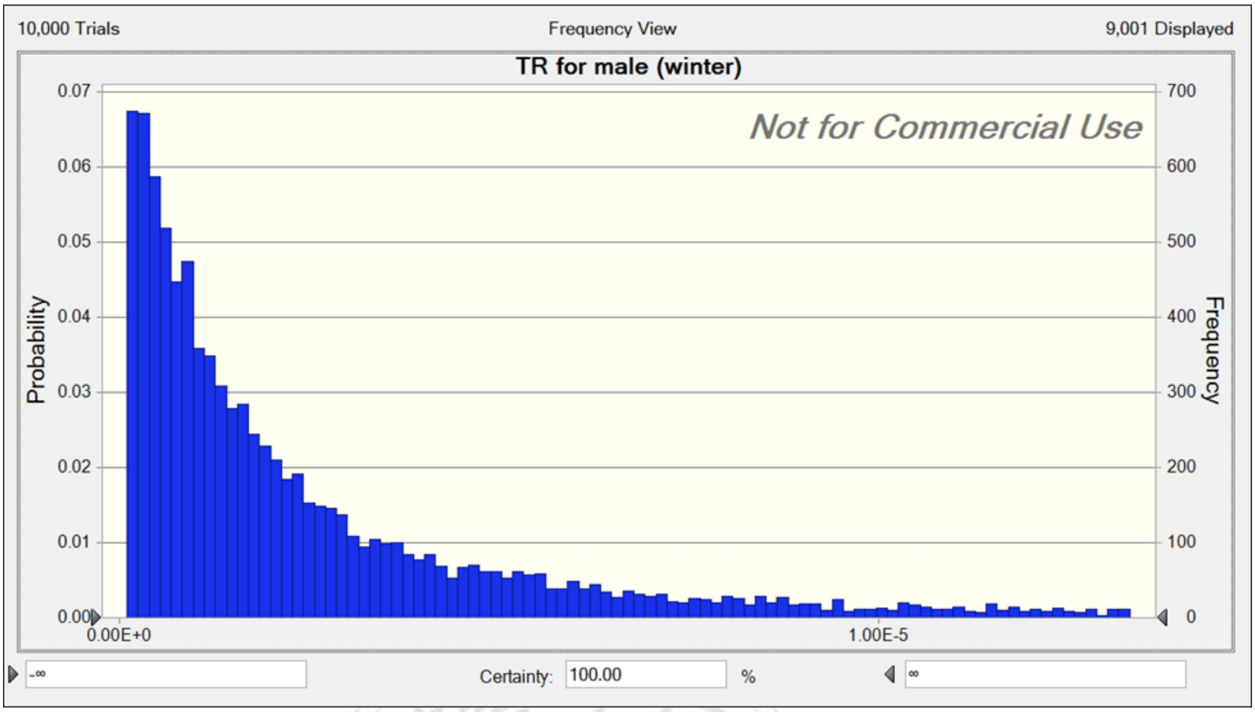

Figure 15: Target Risk graph for male (winter)

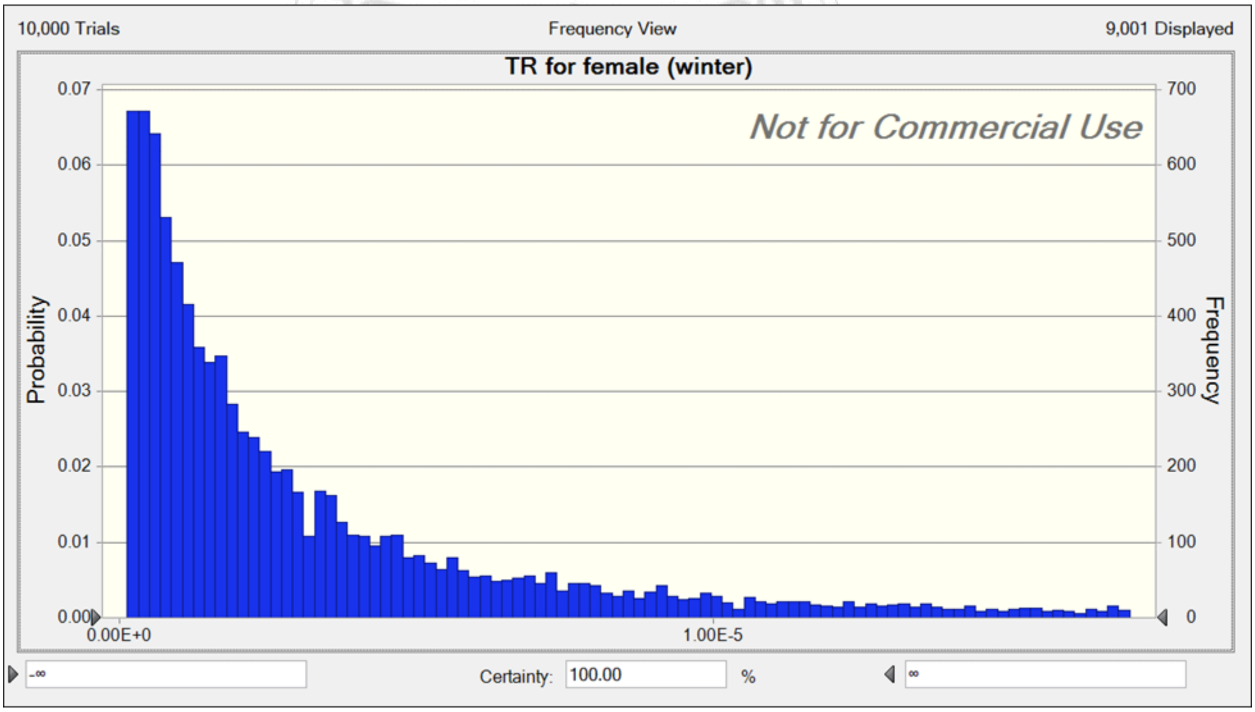

Figure 16: Target Risk graph for female (winter)

Chapter 5
CONCLUSION AND RECOMMENDATION

5.1 Research Conclusion

As per the objective of this study, the total nitrate concentration under the influence of harvesting time (summer and winter), gender (male and female) and the corresponding risk assessment for the possible nitrate exposure was successfully derived. Based on the given values, the total concentration for each vegetable were seperated between the season and gender specific value.

For results gathered, Cveg, DI, HQ, and TR values were higher during the summer season than in winter. In relation to this, most of the data were also high for the female respondent given that they have the high values derived from every parameter mentioned.

Nitrate is already described as a non-carcinogenic chemical based in the IRIS database. In this study, the non-carcinogenic data was described as the values for the HQ, in doing so, the mean value from the HQ computation shows a lower value from the standard value provided by the USEPA, which means that during the average case scenario, HQ is still at its acceptable limit indicating the potential non-cancer risk of nitrate associated with vegetable consumption for Taiwan residents was acceptable. However, during the worst case scenario at the upper 95% value, female respondents was higher during summer time indicating that the non carcinogenic risk fro female during summer time should also be noticed.

In addition, the carcinogenic risk was described from the data derived in TR computation. As a result, all cancer risk (*TR*) value ranged between the negligible level (10^{-6}) and acceptable level (10^{-4}). More so, values derived were higher during summer time than in winter supporting the claims of different authors that higher nitrate concentration are present mostly during summer due to the different factors like the different agricultural products for growing and application of fertilizers. Therefore, the cancer risk of nitrate associated with vegetables consumption should be noticed. In addition, the *DI*, *HQ*, and *TR* values for adult female were higher than those for male, both for summer and winter season vegetable consumption.

5.2 Research Limitation and Future Research Recommendation

This research has certain limitations that is highly suggested to have a future study. The data provided on this research was based on a limited value of parameters such as season, vegetable type, concentration, and gender values. Therefore, results presented on this study cannot represent all Taiwan residents and all types of season and vegetables.

Future research should further study more specific samples that can cover the following:

- different type of vegetables
- the all year round season in Taiwan
- and the different evaluation based on the age for all the respondents involved in the study.

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APPENDIX Table 13.~ Table 18.

Table 13. Vegetables and Nitrate concentrations during summer

NAME OF VEGETABLE	LETTUCE	SPINACH	AMARANTH	CHINESE CABBAGE	WATER SPINACH
	385	2488	4853	884	1890
	688	1721	4733	1057	1520
	1181	4490	5715	1037	3053
	707	1392	5234	986	2758
	1152		4625	984	2256
	3010		2940	2945	2321
			2609	4236	1912
				3691	
NUMBER OF SAMPLES	6	4	7	8	7
				Total number of samples	32

NAME OF VEGETABLE	SWEET POTATO LEAVES	HYDROPONIC CABBAGE	ORGANIC PECHAY	PECHAY	ROMAINE "COASTAL STAR" LETTUCE
	980	6500	2573	2800	985
	2230	4852	7321	2208	360
	1464	9193			317
	715				
	4944				
	5097				
	732				
	720				
	654				
NUMBER OF SAMPLES	9	3	2	2	3
				Total number of samples	19

NAME OF VEGETABLE	ORGANIC PAK - CHOI	PAK-CHOI	ORGANIC GREEN MUSTARD	ORGANIC AMARANTH	MALABAR SPINACH
	5256	6467	3850	6600	985
	5384	6997	6750	5900	1217
			5564		1180
NUMBER OF SAMPLES	2	2	3	2	3
				Total number of samples	12

NAME OF VEGETABLE	RAPE	CHINESE CABBAGE (spp)	YELLOW ZUCCHINI	GREEN ZUCCHINI	CARROT
	2808	5020	980	850	250
			856	877	
NUMBER OF SAMPLES	1	1	2	2	1
				Total number of samples	7

NAME OF VEGETABLE	WHITE RADISH	CABBAGE TURNIP	CELERY	ORGANIC CANOLA	ORGANIC SPINACH
	740	330	302	1500	100
NUMBER OF SAMPLES	1	1	1	1	1
				Total number of samples	5

NAME OF VEGETABLE	GREEN PEPPER	RADISH	TOMATO	COW TOMATO	ORGANIC PAK-CHOI (spp)
	88	980	80	120	862
	25		44	35	
NUMBER OF SAMPLES	2	1	2	2	1
				Total number of samples	8
				Overall total of samples	83

Table 14. Monte Carlo Simulation results for summer (male)

Statistics	DI	HQ	TR	BW	Cveg	IR
Trials	10000	10000	10000	10000	10000	10000
Base Case	---	---	---	0	0	0
Mean	0.397556	0.248472	3.98E-06	72.51458	2664.219	9.972926
Median	0.228985	0.143115	2.29E-06	72.35565	1670.003	9.811498
Mode	---	---	---	---	---	---
Standard Deviation	0.559286	0.349554	5.59E-06	18.02294	3292.475	1.996504
Variance	0.3128	0.122188	3.13E-11	324.8263	10840394	3.986029
Skewness	4.92661	4.92661	4.92661	0.037118	3.679493	0.630751
Kurtosis	46.09269	46.09269	46.09269	3.085886	24.56532	3.942008
Coeff. of Variation	1.40681	1.40681	1.40681	0.248542	1.235813	0.200192
Minimum	-0.11302	-0.07064	-1.1E-06	3.568002	-179.57	4.673687
Maximum	11.36667	7.104167	0.000114	145.5353	43108.09	23.64874
Range Width	11.47969	7.174803	0.000115	141.9673	43287.66	18.97505
Mean Std. Error	0.005593	0.003496	5.59E-08	0.180229	32.92475	0.019965
Percentiles	**DI**	**HQ**	**TR**	**BW**	**Cveg**	**IR**
2.5%	0.007503	0.004689	7.5E-08	37.50392	61.38866	6.618708
25%	0.102097	0.06381	1.02E-06	60.51349	759.5596	8.55903
50%	0.22894	0.143087	2.29E-06	72.35309	1669.751	9.811187
95%	1.327124	0.829453	1.33E-05	102.378	8454.969	13.51402
97.5%	1.82799	1.142494	1.83E-05	108.3737	11710.07	14.34891
Sensitivity Data						
Assumptions	**DI**	**HQ**	**TR**			
BW	-0.21552	-0.21552	-0.21552			
Cveg	0.952212	0.952212	0.952212			
IR	0.175419	0.175419	0.175419			

Table 15. Monte Carlo Simulation results for summer (female)

Statistics	DI	HQ	TR	BW	Cveg	IR
Trials	10000	10000	10000	10000	10000	10000
Base Case	---	---	---	0	0	0
Mean	0.516787	0.322992	5.17E-06	58.60833	2651.427	10.03011
Median	0.283284	0.177052	2.83E-06	58.47301	1626.742	9.853954
Mode	---	---	---	---	---	---
Standard Deviation	1.118747	0.699217	1.12E-05	17.38229	3307.178	2.015798
Variance	1.251596	0.488905	1.25E-10	302.1441	10937423	4.063442
Skewness	30.98382	30.98382	30.98382	0.021099	3.860624	0.583438
Kurtosis	2181.357	2181.357	2181.357	2.979372	28.45603	3.504006
Coeff. of Variation	2.164814	2.164814	2.164814	0.296584	1.24732	0.200975
Minimum	-30.3031	-18.9395	-0.0003	-7.43051	-213.101	4.152944
Maximum	76.29804	47.68627	0.000763	133.1032	53189.38	19.48082
Range Width	106.6012	66.62574	0.001066	140.5337	53402.49	15.32788
Mean Std. Error	0.011187	0.006992	1.12E-07	0.173823	33.07178	0.020158
Percentiles	DI	HQ	TR	BW	Cveg	IR
2.5%	0.007663	0.00479	7.66E-08	24.40861	51.16615	6.628489
25%	0.125646	0.078529	1.26E-06	46.69563	759.5212	8.590285
50%	0.283235	0.177022	2.83E-06	58.46805	1626.734	9.853924
95%	1.732584	1.082865	1.73E-05	87.34338	8554.703	13.64203
97.5%	2.407884	1.504927	2.41E-05	92.45412	11503.53	14.57203
Sensitivity Data						
Assumptions	DI	HQ	TR			
BW	-0.2742	-0.2742	-0.2742			
Cveg	0.934919	0.934919	0.934919			
IR	0.161784	0.161784	0.161784			

Table 16. Vegetables and Nitrate concentrations during winter

NAME OF VEGETABLE	LETTUCE	SPINACH	AMARANTH	CHINESE CABBAGE	WATER SPINACH
	332	3763	5502	1024	3295
	177	2293	4987	77	3780
	223	4560	2198	1041	3996
	504	4732			347
	1285	4627			26
	1444	2859			
	1954	3873			
	456	2487			
	437	2772			
		2542			
		3416			
		1311			
		1792			
NUMBER OF SAMPLES	9	13	3	3	5
				Total number of samples	33

NAME OF VEGETABLE	SWEET POTATO LEAVES	HYDROPONIC CABBAGE	PECHAY	ROMAINE "COASTAL STAR" LETTUCE	BOK-CHOI
	1368	5843	2940	1203	5855
	1054	6058	4157	313	4759
			4497	1243	2555
			2539	1225	895
			2555	1186	1567
			2732	650	
			2779	2760	
			868		
			1194		
			1350		
			370		
			705		
			1884		
NUMBER OF SAMPLES	2	2	13	7	5
				Total number of samples	29

NAME OF VEGETABLE	CABBAGE	ROMAINE LETTUCE	KALE	CHRYSANTHEMUM LEAVES	RAPE
	395	2333	2940	2706	3998
	272	2167	1194		2947
	376	2125			2754
		1589			
NUMBER OF SAMPLES	3	4	2	1	3
				Total number of samples	13

NAME OF VEGETABLE	CHINESE CABBAGE (spp)	YELLOW ZUCCHINI	GREEN ZUCCHINI	SMALL CHINESE CABBAGE	NAPA CABBAGE
	5184	1052	1073	2887	770
	4928				
	4915				
	2617				
	3833				
NUMBER OF SAMPLES	5	1	1	1	1
				Total number of samples	9

NAME OF VEGETABLE	CHRYSANTHEMUM LEAVES (spp)	BROCCOLI	BROCCOLI (spp)	WHITE RADISH	ROMAINE LETTUCE (spp)
	914	26	55	572	233
	622	128	85		219
		188	64		
NUMBER OF SAMPLES	2	3	3	1	2
				Total number of samples	11

NAME OF VEGETABLE	CABBAGE TURNIP	CELERY	FENNEL	TOMATO	COW TOMATO
	384	463	1749	55	28
	483			49	32
NUMBER OF SAMPLES	2	1	1	2	2
Total number of samples					8
Overall total of samples					103

Table 17. Monte Carlo Simulation results for winter (male)

Statistics	**DI**	**HQ**	**TR**	**BW**	**Cveg**	**IR**
Trials	10000	10000	10000	10000	10000	10000
Base Case	---	---	---	0	0	0
Mean	0.319919	0.199949	3.2E-06	72.70386	2138.032	10.00725
Median	0.177857	0.111161	1.78E-06	72.83116	1272.675	9.829509
Mode	---	---	---	---	---	---
Standard Deviation	0.482701	0.301688	4.83E-06	18.05613	2775.445	1.981079
Variance	0.233	0.091016	2.33E-11	326.0238	7703095	3.924675
Skewness	6.933037	6.933037	6.933037	-0.04288	4.278585	0.595269
Kurtosis	114.4018	114.4018	114.4018	2.957012	35.45521	3.672382
Coeff. of Variation	1.508823	1.508823	1.508823	0.248352	1.298131	0.197964
Minimum	-0.04185	-0.02616	-4.2E-07	9.918042	-171.857	4.752105
Maximum	14.62845	9.142783	0.000146	140.8177	45589.99	23.70101
Range Width	14.6703	9.168939	0.000147	130.8997	45761.84	18.9489
Mean Std. Error	0.004827	0.003017	4.83E-08	0.180561	27.75445	0.019811
Percentiles	**DI**	**HQ**	**TR**	**BW**	**Cveg**	**IR**
2.5%	0.003084	0.001927	3.08E-08	37.36632	24.25007	6.667626
25%	0.077292	0.048308	7.73E-07	60.45413	573.4034	8.609402
50%	0.177808	0.11113	1.78E-06	72.82577	1272.566	9.828752
95%	1.085418	0.678386	1.09E-05	102.1032	6977.186	13.53445
97.5%	1.454202	0.908876	1.45E-05	107.7574	9272.159	14.38158
Sensitivity Data						
Assumptions	**DI**	**HQ**	**TR**			
BW	-0.20446	-0.20446	-0.20446			
Cveg	0.955373	0.955373	0.955373			
IR	0.149775	0.149775	0.149775			

Table 18. Monte Carlo Simulation results for winter (female)

Statistics	DI	HQ	TR	BW	Cveg	IR
Trials	10000	10000	10000	10000	10000	10000
Base Case	---	---	---	0	0	0
Mean	0.41896	0.26185	4.19E-06	58.26899	2140.544	9.991546
Median	0.219212	0.137007	2.19E-06	58.39847	1273.07	9.786624
Mode	---	---	---	---	---	---
Standard Deviation	0.7001	0.437563	7E-06	17.21844	2896.233	1.995949
Variance	0.49014	0.191461	4.9E-11	296.4748	8388167	3.983812
Skewness	9.838418	9.838418	9.838418	-0.0024	5.726458	0.610121
Kurtosis	240.1803	240.1803	240.1803	2.984237	83.51104	3.69275
Coeff. of Variation	1.671045	1.671045	1.671045	0.295499	1.353036	0.199764
Minimum	-4.6659	-2.91619	-4.7E-05	-8.67113	-192.207	4.701648
Maximum	26.03672	16.27295	0.00026	131.8964	80244.34	20.50049
Range Width	30.70262	19.18914	0.000307	140.5675	80436.55	15.79884
Mean Std. Error	0.007001	0.004376	7E-08	0.172184	28.96233	0.019959
Percentiles	DI	HQ	TR	BW	Cveg	IR
2.5%	0.002981	0.001863	2.98E-08	24.3151	22.5294	6.638994
25%	0.093231	0.05827	9.32E-07	46.59678	567.041	8.583466
50%	0.219175	0.136984	2.19E-06	58.39383	1273.066	9.786507
95%	1.449262	0.905789	1.45E-05	86.42482	6905.003	13.57079
97.5%	2.01387	1.258669	2.01E-05	92.34534	9557.714	14.42358
Sensitivity Data						
Assumptions	DI	HQ	TR			
BW	-0.25591	-0.25591	-0.25591			
Cveg	0.943285	0.943285	0.943285			
IR	0.173036	0.173036	0.173036			