UNIVERSITI PUTRA MALAYSIA

DETERMINATION OF CADMIUM CONTAMINATION IN CANNED FISH AND HEALTH RISK ASSESSMENT AMONG STUDENTS OF FACULTY OF MEDICINE AND HEALTH SCIENCES, UNIVERSITI PUTRA MALAYSIA

BY
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ABSTRACT

DETERMINATION OF CADMIUM CONTAMINATION IN CANNED FISH AND HEALTH RISK ASSESSMENT AMONG STUDENTS OF FACULTY OF MEDICINE AND HEALTH SCIENCES, UNIVERSITI PUTRA MALAYSIA

LISA PUTRIKU DIANA BINTI AB KAHAR

Introduction: Heavy metal such as cadmium can be highly toxic even at low doses when ingested over a long time period. In Malaysia, canned fish product were manufactured either locally or imported and this food is very popular in supermarkets and groceries retail outlet. This study is a cross-sectional study that was conducted at 17 College, UPM. **Objective:** The aim of this study was to determine cadmium concentration level in the canned fish consume by the respondents. The study was also to determine their potential detrimental effects via calculation of the LADD, ADD, HQ and LCR for average daily consumption of these canned fish. **Material and Methodology:** A total of 215 respondents involved in this study. A set of pre-tested questionnaire was used to obtain socio-demographic information and to predict the health risk faced by respondents which indicated by ADD, LADD, frequency canned fish intake, health symptoms and disease experienced by respondents. In sample analysis, dry ashing method was used for the extraction of the samples and ICP-MS was used to determined cadmium concentration level in the samples. **Result:** The result showed the range of cadmium concentration in canned fish samples were between 0.0026 to 0.0192 mg/kg did not exceeded Malaysia Food Act 1983 standard for cadmium (1 mg/kg). The mean value of ADD was $2.357 \times 10^{-6} \pm 3.8206 \times 10^{-6}$ mg/kg/day and $3.437 \times 10^{-7} \pm 6.217 \times 10^{-7}$ mg/kg/day for LADD which did not exceed the limit for PTWI. The LCR showed all respondents encountered clearly acceptable risk to carcinogen health effect and had HQ less than 1. There was a significant relationship ($p = 0.0001$) between frequency intake of canned fish and health risk encountered for both canned sardine and tuna spread. **Conclusion:** As conclusion, high frequency intake of canned fish might gives risk on respondents health. Proper dietary intake of food can help reducing the absorption of cadmium into body. Further assessment is needed to control the risk of heavy metals in dietary intake. More study on canned food and seafood quality are encouraged, to provide the data and guideline for human health in Malaysia.

Keywords: Students, Canned fish, Cadmium, ICP-MS, Provisional Tolerable Weekly Intake (PTWI)

ABSTRAK

PENENTUAN PENCEMARAN KADMIUM DALAM IKAN DALAM TIN DAN PENILAIAN RISIKO DAN KESIHATAN DI KALANGAN PELAJAR FAKULTI PERUBATAN DAN SAINS KESIHATAN, UNIVERSITI PUTRA MALAYSIA

LISA PUTRIKU DIANA BINTI AB KAHAR

Pengenalan: Logam berat seperti kadmium boleh menjadi sangat toksik walaupun dimakan pada dos yang rendah dalam tempoh jangka masa yang panjang. Di Malaysia, produk ikan dalam tin adalah dikilangkan samaada secara tempatan atau diimport dan makanan ini sangat popular di pasaraya dan kedai runcit. Kajian ini adalah kajian keratan rentas yang telah dijalankan di Kolej 17, UPM. **Objektif:** Tujuan kajian ini adalah untuk menentukan tahap kepekatan kadmium dalam ikan dalam tin. Kajian ini juga adalah untuk menentukan kesan pengambilan terhadap kesihatan melalui pengiraan *LADD, ADD, HQ* dan *LCR* daripada pengambilan purata harian ikan dalam tin ini. **Bahan dan Kaedah:** Seramai 215 responden terlibat dalam kajian ini. Satu set soalan pra-uji digunakan untuk mendapatkan maklumat sosio-demografi dan untuk meramal risiko kesihatan yang dihadapi oleh responden yang ditunjukkan oleh *ADD, LADD,* kekerapan pengambilan ikan dalam tin, kesan-kesan pada kesihatan yang dialami oleh responden. Dalam analisis sampel, kaedah pengabuan kering digunakan untuk pengekstrakan sampel dan ICP-MS telah digunakan untuk menentukan tahap kepekatan kadmium di dalam sampel. **Keputusan:** Hasil kajian menunjukkan julat kepekatan kadmium di dalam sampel ikan dalam tin adalah diantara 0.0026 hingga 0.0192 mg / kg tidak melebihi standard Akta Makanan Malaysia 1983 untuk kadmium (1 mg / kg). Nilai min *ADD* adalah $2.357 \times 10^{-6} \pm 3.8206 \times 10^{-6}$ mg / kg / hari dan $3.437 \times 10^{-7} \pm 6.217 \times 10^{-7}$ mg / kg / hari untuk *LADD* tidak melebihi had *PTWI*. *LCR* menunjukkan semua responden tidak menghadapi risiko kesihatan karsinogen dan mempunyai *HQ* kurang daripada 1. Terdapat signifikasi perhubungan ($p = 0.0001$) diantara pengambilan kekerapan ikan dalam tin dan risiko kesihatan yang dihadapi bagi kedua-dua ikan sardin dan ikan tuna dalam tin. **Kesimpulan:** Kekerapan pengambilan ikan dalam tin yang tinggi mungkin memberikan risiko pada kesihatan responden. Penilaian lanjut diperlukan untuk mengawal risiko logam berat dalam pengambilan makanan. Lebih banyak kajian pada makanan dalam tin dan kualiti makanan laut adalah digalakkan untuk menyediakan data dan garis panduan untuk kesihatan manusia di Malaysia.

Keywords: Pelajar, ikan dalam tin, kadmium, ICP-MS, PTWI

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LIST OF ABBREVIATIONS

Cd	Cadmium
DNA	Deoxyribonucleic acid
EPA	Ecosapentaenoic acid
DHA	dicosahexanoic acid
RfD	Reference Dose
EPA	Environmental Protection Agency
FAO	Food and AgricultureOrganization
WHO	World Health Organization
PTWI	Provisional Tolerable Weekly Intake
IRIS	Integrated Risk System
ATSDR	Agency for toxic Substances and Disease Registry
ICP-MS	Inductively Coupled Plasma-Mass Spectrometry
ADD	Average Daily Dose
LADD	Lifetime Average Daily Dose
HQ	Hazard Quotient
LCR	Lifetime Excess cancer
CSF	Cancer Slope Fcctor
BMI	Mass Body Index

CHAPTER 1

INTRODUCTION

1.1 Background

Pollution of heavy metal cadmium had recognized as a worldwide public health hazard. It was persistence in the environment and its very long biological half-life 10-40 years in human body (Rubio, 2005). Cadmium was a soft, bluish-white metallic (Puneet et al., 2010) element that occurs naturally in the earth's crust. The physical property of cadmium was atomic number-48, atomic weight-112.411, electro-negativity-1.5, crystal ionic radius (Principal valence state)-0.97, ionisation potential-8.993, oxidation state +2, electron configuration Kr 4d1 5S2, density-8.64 g/cm3, melting point - 320.9°C and boiling point-765°C at 100 kPa (Puneet et al,, 2010). Cadmium was often found as a mineral combined with other elements like oxygen (cadmium oxide), chlorine (cadmium chloride), or sulphur (cadmium sulphate, cadmium sulphide) (Puneet et al., 2010). Its surface had a bluish tinge and the metal was soft enough to be cut with a knife, but it tarnishes in air. Cadmium was soluble in acids but not in alkalis. It was similar in many respects to zinc but it forms more complex compounds. Other than that, cadmium usually occurs as a minor component in most zinc ores and therefore it was a byproduct of zinc production. Cadmium was also used for a long time as a pigment and for corrosion resistant plating on steel while cadmium compounds were used to stabilize plastic.

Industrialization had improved general technology as well as quality of life but had also resulted in an increase in metal concentration in water (Ufuk, 2007). Cadmium can be release into environment by human activities such as in industrial applications; electroplating, pigment production, manufacture of plastic stabilizers and pigments, nickel-cadmium batteries and electronics and others. Contamination of cadmium can also occur during food processing or packaging (Ashraf et al, 2006). While, fertilizers produced from phosphate ores, industrial operations such as mining, mining refining and heavy metals release into the rivers and seas were other important sources of environmental contamination.

In 1968, the Ministry of Health and Welfare in Japan officially admitted that 'itai-itai' disease was caused by primarily by cadmium (Rubio, 2005). In those 'itai-itai' episode, ingested excess amounts induces toxicity symptoms in man include gastrointestinal pains, nausea, respiratory distress, diarrhea, impaired reproductive, kidney damage and hypertension (Ashraf et al., 2006). Since this tragic food poisoning happen, there have been growing interest in assessing the level of toxic heavy metals in food (Ashraf et al., 2006).

Cadmium can enter our body through eating, drinking or breathing. Some cadmium stays in our body, some was breathed out, and some leaves the body as waste. Most of the cadmium in our body was stored in our kidney and liver. It can stay there for many years. Our body can change cadmium into a harmless form. However, too much cadmium can make it difficult for our liver and kidneys to process it and that will leads to dangerous health effects. Breathing high levels of cadmium can cause death and severe lung damage. Eating food or drinking water with very high levels of cadmium can hurt the stomach such as causing vomiting or diarrhoea. While, long-term exposure to lower levels of cadmium may cause kidney disease, lung damage and fragile bones.

Other health effects that can be caused by cadmium were such as reproductive failure and possibly even infertility, damage to the central nervous system, damage to the immune system, psychological disorders, possibly DNA damage or cancer development such as pancreatic cancer. Gary (2000) says that cadmium can cause transdifferentiation of pancreatic cells, which will increases in the synthesis of pancreatic DNA, and this will increases in oncogene activation. Thus, cadmium is a plausible pancreatic carcinogen. Moreover, the Department of Health and Human Services (DHHS) of ASTDR has determined that cadmium and cadmium compounds are known human carcinogens.

Canned foods were popular in developing countries and canned fishes were one of the most popular foods (Voegborlo et al., 1999; Ikem and Egeibor, 2005; Ashraf et al., 2006; Khansari et al., 2005; Suhendan, 2010). Canned food offer a shortcut in preparing meal which was more favorable, simple and easy by those who were lack of time in preparing their meal. In Malaysia, most of the manufacturing canned sardine's products were from Malaysia and Thailand. Canned fish, such as canned sardines, canned tuna product manufactured either locally or imported, very popular in supermarkets and groceries retail outlet (Abdul, 2009).

Most fish have less fat than most meats per serving, and the fats in fish were less saturated. For example, a 4-ounce serving of baked bluefish had 6 g fat (4.1 g saturated fat) and 86 mg cholesterol; a 4-ounce serving of lean sirloin had 9 g fat (4.7 g saturated fat), and 101 mg cholesterol. The most prominent fats in fish were omega-3 fatty acids: ecosapentaenoic acid (EPA), dicosahexanoic acid (DHA), and the essential fatty acid linolenic acid. Omega-3s, also found in human breast milk, were most abundant in fish living in cold waters (anchovy, herring, mackerel, menhaden, salmon, sardines, trout, and tuna). The safety issues related to the possibility of heavy metal were of concern nowadays. Throughout from the previous episode on cadmium poisoning in fish contamination, fish were acknowledged to be the single largest source of cadmium for man.

1.2 Problem Statement

The metal cadmium was a relatively rare element and was not found in the pure state in nature. Cadmium had a limited number of applications but was used for a variety of consumer and industrial materials. From time to time episodes of acute cadmium poisoning were reported, emphasizing the potential toxicity of this metal and its salts. Human cadmium toxicity caused by contaminated rice plants was first reported in Japan in the 1950s. These studies showed that subsistence rice farmers had been sickened by ingesting cadmium that had passed from municipal sewage sludge used as fertilizer through the rice crop. Cadmium was taken up through the roots of plants to edible leaves, fruits and seeds. It will also build up in animal milk and fatty tissues. Therefore, people were exposed to cadmium upon consumption of cadmium containing plants or animals. Seafood, such as fish, mollusks and crustaceans, can be a source of cadmium, as well.

And today, the worries of the effect on the anthropogenic pollution on the marine aquatic ecosystem were rising up (Ashraf et al., 2006). Monitoring toxic metal level in aquatic ecosystem especially fish was the need of the awareness from public health point of view. Heavy metal cadmium was known as non-biodegradable and this will cause accumulation of the heavy metals such as cadmium in marine life especially in fish, thus this will cause the accumulation of heavy metals in the chain food.

In previous study, the maximum tolerable level of cadmium in the kidney, in order to avoid abnormal kidney function, was 50μg/g wet weight (Rubio, 2005). Moreover, the typical daily dietary intake of cadmium was 10 to 20 μg. Examples of significant sources of cadmium include shellfish, grains especially those grown on high cadmium soils and leafy vegetables. Cadmium had a long half-life in the body and thus high intakes can lead to accumulation resulting in damage in some organs, especially the kidney. The Reference Dose (RfD) (safe daily intake over a lifetime) for cadmium was 0.5 μg per kg body weight (Nielsen, 1996).

Other than that, cadmium had been reported to exert deleterious effects in terms of nephrotoxic, cytotoxic, genotoxic, immunotoxic and carcinogenic (Singh, 2010), only 3% - 5% from food dietary intake of cadmium in food will be absorb in our body. Ingestion of foods that contain cadmium can cause resulting in abdominal pain, nausea, and vomiting. Cadmium in body usually accumulates in kidney. This can cause renal tubular damage because of the inability of the kidneys to effectively filter cadmium and this was an irreversible damage on kidney. Cadmium in the kidneys, causing excess calcium to be excreted instead of absorbed, may affect the mineralization of bone. Clinical evidence of the cumulative effects of cadmium did not usually appear until well after exposure. The Environmental Protection Agency (EPA) had classified cadmium as a probable human carcinogen.

1.3 Study Justification

Nowadays, almost all natural water was contaminated with lots of types of heavy metals. Our marine aquatic life also endangered not only from the effect of the natural heavy metals in environment but also from human activities. In globally, fish was type of food that becoming the main source of protein to human. Food and Agriculture Organization (FAO) and World Health Organization (WHO) (1998) had estimated that around 15% - 20% of our food source of protein was from fish. But, fish was also found to be the source possibility of contamination of heavy metals and its risk on human.

The study location for this study was chosen because of the majority of the students live in the college provided and being independent to survive living in college by themselves. There were high possibilities that their daily food intake will be depending on process food. Other than that, all the populations were from health courses and with this, it was assumed that the students should have high awareness of the presents of heavy metals in canned food and the health effect on human.

In Malaysia, 23% of our source of protein was from fish (Tukimat et al., 2005). In Malaysia, there was still lack of study on the amount of heavy metal of cadmium in canned food. With that, exposure prevention to canned fish that have contamination in food can be practice to reduce the mortality rate or morbidity rate among community population in Malaysia that cause by diseases that comes from cadmium toxication. In Malaysia Food Act 1983, level dose of cadmium allows in marine aquatic food was 1μg/L, for daily intake was 0.064mg and the death dose of cadmium was 10mg. Other than that, World Health Organisation (WHO) had established a provisional tolerable weekly intake (PTWI) for cadmium at 7 μg/kg of body weight. This PTWI weekly value corresponds to a daily tolerable intake level of 70 μg of cadmium for the average 70-kg man and 60 μg of cadmium per day for the average 60-kg woman. This study was to determine the cadmium level in canned fish food and to estimate the health risk of cadmium to human exposure.

1.4 Conceptual Framework

Figure 1 shown was the conceptual framework of steps and aspect that will be study in this study. This study was to determine cadmium contamination in canned fish and health risk assessment among students of Faculty of Medicine and Health Sciences, Universiti Putra Malaysia. In this study, place to buy the sample of canned fish and type of canned fish will be depending on the most preferable choice from the respondents.

There were two type of source for cadmium that can be found in the environment which was from the natural source and also from manmade or man activities. As we know nowadays the pollution of heavy metals in water had greatly contaminated our environment. Through water source was place which was one of the main source of protein such as fish and because of human activities, cadmium have been accumulating in our food chain; fish that had being contaminated will accidentally being consumed in our daily life dietary intake. The fish might had expose to excessive cadmium in environment such from oil spill in the sea by big ship, animals waste and many more.

In this study, we will study on cadmium contamination in canned fish. Cadmium that being consume through contaminate canned fish will absorb into the respondents body through the ingestion and next will goes through the body blood supply and then will distribute to the whole body and organs especially in liver and kidney where cadmium will accumulate the most. In kidney, the re-absorption process for cadmium waste and also protein will be less efficient. The waste materials in kidney will get more and more because of unable to be re-absorbed again into the body. When this condition was going through for a long period of time, it will cause damage to kidney and cause kidney failure.

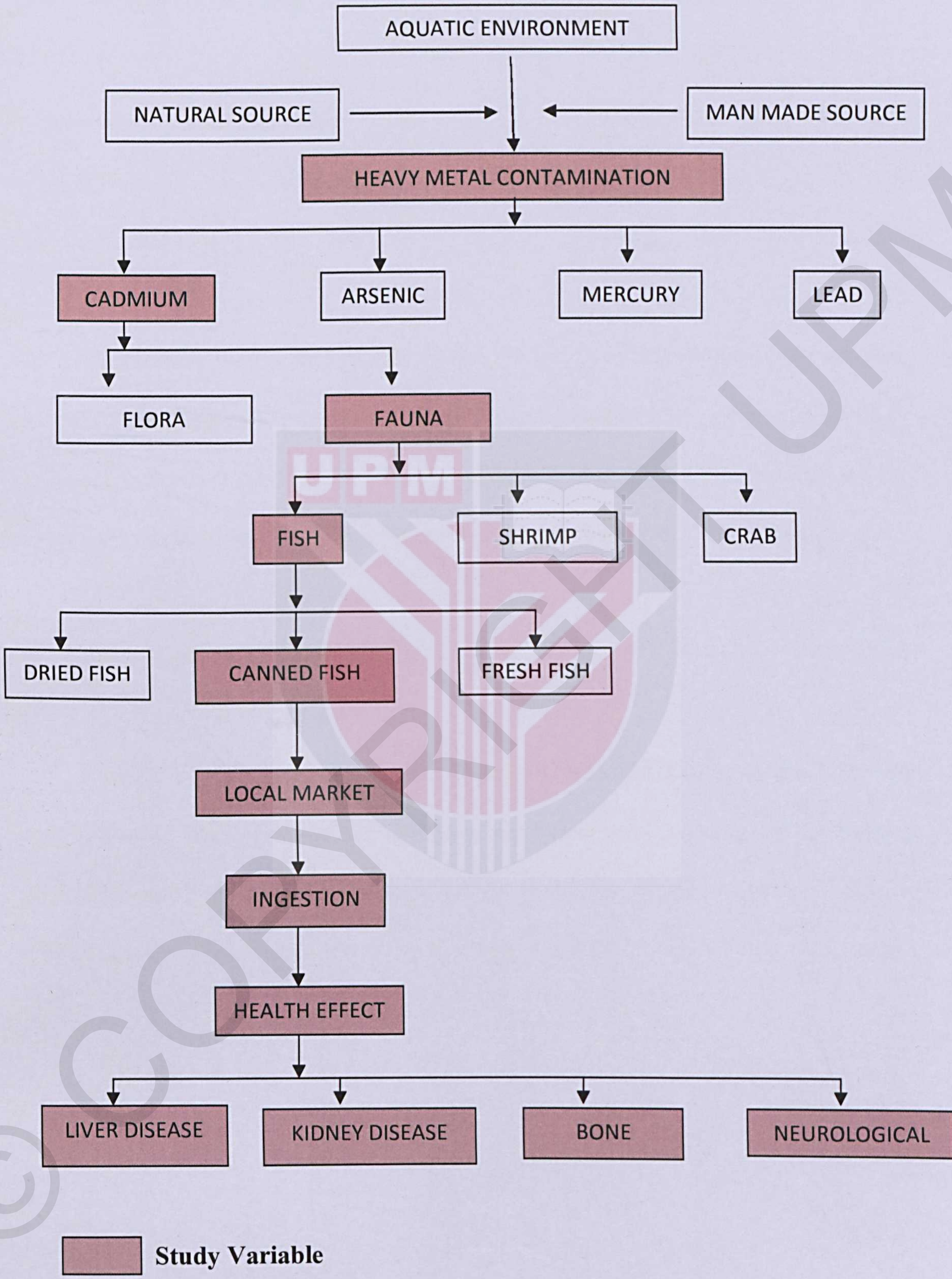


Figure 1: Conceptual Framework

1.5 Definition of Term

1.5.1 Conceptual Definition

a. Cadmium

Cadmium was a widespread non-essential toxic heavy metal, which was known to affect cellular processes through membrane damage, disruption of electron transport, enzyme inhibition or activation and DNA alteration. Cadmium was highly toxic even at low doses when ingested over a long time period (Ufuk, 2007).

b. Cadmium in canned fish

Heavy metals such as cadmium in canned fish, depending on the type and also origin of the food, the pH of the canned food product, amount of oxygen concentration in the headspace, the quality of the inside lacquer coating of cans, it storage condition and other factors that might affect the canned food (Tarley et al., 2001).

c. **Health Risk Assessment (HRA)**

Health risk assessment was a scientific tool designed to determine particular hazard poses a significant risk to human health and, if so, under what circumstances. Risk assessment helps scientists and regulators identify serious health hazards and determine realistic goals for reducing exposure to toxics so that there was no significant health threat to the public (California Environmental Protection Agency, Office of Environmental, Health Hazard Assessment).

Quantitative health risk assessment should contain some or all the 4 steps, namely hazard identification, dose-response assessment, exposure assessment and risk characterization. The application of quantitative health risk assessment was mainly limited to the assessment of hazard.

Hazard Identification was information about a potential hazard diligently seek in order to properly compile adequate information about the hazard. Hazard identification evidence about the hazard may be source from local as well as foreign epidemiological studies. Other than that, it can also be extracted from scientifically reliable database such as the U.S. EPA's Integrated Risk System (IRIS), and Agency for toxic Substances and Disease Registry's (ATSDR's) Toxic Substances Portal.

Dose-Response Assessment was a dose response-relationship to describe the increase in the probability of an adverse effect with a corresponding increase in the exposure dose to the hazard in question. The reference dose (RfD) was an estimated daily oral exposure of a toxicant in µg/kg/day, with uncertainty spanning perhaps an order of magnitude, to human population including sensitive subgroups, that was likely to be without an appreciable risk of deleterious effect during a lifetime of 70 years.

Exposure Assessment had two main component of risk which was hazard and exposure. Base on the dose-response assessment, if the exposure dose was known or can be predicted, we can describe the adverse effect that will be manifested. But, if exposure had already taken place, we can measure the exposure and correlate it with the health effect outcome. And when exposure was yet to be experience, then the adverse effect will have to be predicted through health risk assessment.

1.5.2 Operational Definition

a. Cadmium

Cadmium was a byproduct of the mining and smelting of lead and zinc. It was used in nickel-cadmium batteries, PVC plastics, and paint pigments. It can be found in soils because insecticides, fungicides, sludge, and commercial fertilizers that use cadmium were used in agriculture. Cadmium may be found in reservoirs containing shellfish. Cigarettes also contain cadmium. Around 2% - 7% of ingested cadmium was absorbed in the gastrointestinal system. Target organs were the liver, placenta, kidneys, lungs, brain, and bones.

b. Cadmium in canned fish

Heavy metal was receiving increasing popularity in food industry due to high incidence of contamination in agricultural and seafood products. Apart from the threat from polluted environment, canned food was subjected to heavy metal contamination during the canning process. The ingestion of food was an obvious means of exposure to metals, not only because many metals were natural components of food stuffs, environmental contamination and contamination during processing but also lack of hygiene while handling food. Cadmium in canned fish can be detected by using ASH extracting method and also by using ICP-MS analysis method.

c. Health Risk Assessment

A health risk assessment also referred to as a health risk appraisal and health and well-being assessment was one of the most widely used screening tools in the field of health promotion and was often the first step in multi-component health promotion programs. Below were health risk assessment procedures.

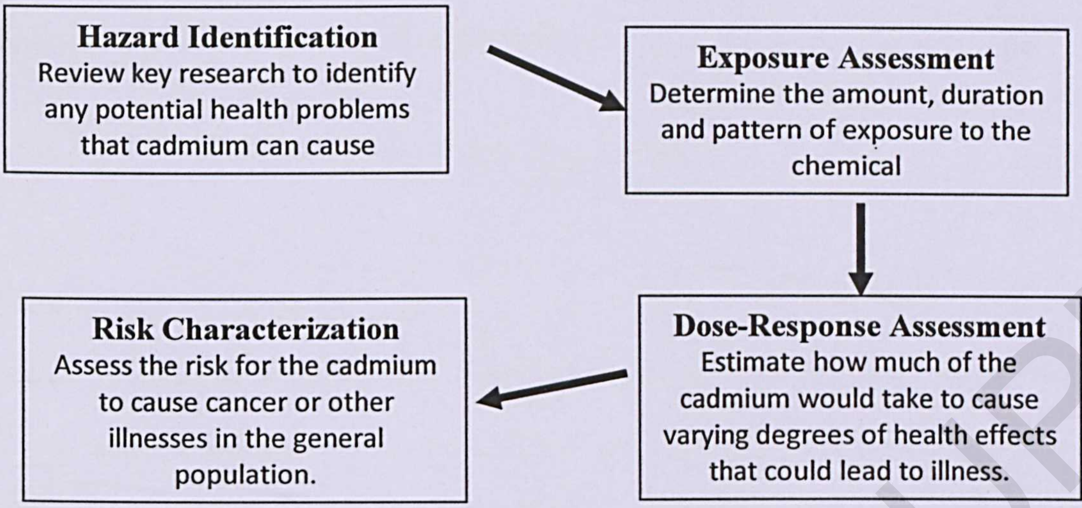


Figure 2: Health Risk Assessment Procedures

The health risk assessment of the respondents was indicated by the average daily dose (ADD) and Hazard Quotient (HQ) for non-carcinogenic health risk. Lifetime Average Daily Dose (LADD) and Lifetime cancer risk (LCR) indicated the carcinogenic health risk. In order to estimate the non-carcinogenic health risk, at first, the ADD and LADD was calculated by using the following equation I and II. (Saipan, 2009).

$$\text{(I)}\quad \text{ADD (mg/kg-day)} = \frac{Cp \times IR \times Ed \times EF}{BW \times AT_{NC}}$$

Where,

ADD = Average daily dose (mg/kg-day) for non-carcinogenic health effect, Cp=Average concentration in the shrimp paste (mg/g), IR=Food Ingestion rate (g/day), Ed= Exposure Duration (yr), EF= Exposure Frequency, BW=Body weight (kg), AT_{NC}=Averaging time (EDx365days/yr)

(II) $$\text{LADD (mg/kg-day)} = \frac{Cp \times IR \times Ed \times EF}{BW \times AT_C}$$

Where,

LADD = Lifetime Average daily dose (mg/kg-day) for carcinogenic health effect, Cp=Average concentration in the shrimp paste (mg/g), IR=Food Ingestion rate (g/day), Ed= Exposure Duration (yr), EF= Exposure Frequency, BW=Body weight (kg), AT_C=Averaging time (25,550 days/yr)

For health risk assessment, non-carcinogenic risk was express by using the hazard quotient (HQ). For ingestion exposure, HQ was ratio of the average daily dose (ADD) to the reference dose (RfD). The exposure of cadmium on respondents was ingesting canned fish which the canned fish might already contaminate to high amount of cadmium in sea. Hazard quotient (HQ) was calculated by using formula;

Estimation of risk for non-carcinogens

$$\text{Hazard Quotient (HQ)} = \frac{\text{ADD}}{\text{Oral RfD}}$$

Where:

Hazard Quotient = Non-cancer Hazard Index of a health effect from intake of cadmium

ADD = Average daily dose (mg/kg/day)

Oral RfD = Oral Reference dose of cadmium (mg/kg/day)

RfD Cd – 5.0 x 10^{-4} μg Cd per kg body weight per day. (Nielsen, 1996).

The calculated HQ value was compared to a benchmark where a HQ value of 1.0 or less than 1.0 indicates that no adverse effects or noncancerous were expected to occur although values less than 1.0 may not be risk free (IRIS, 2009), whereas HQ values greater than 1 indicate a concern for adverse health effects or the need for further study.

While for carcinogenic risk was express by using the estimation life-time risk by using the formula calculation as below;

Estimation of risk for carcinogen

Lifetime Excess Cancer Risk (LCR) = LADD x q*

Where:

LCR = Upper limit cancer risk

LADD = Lifetime average daily dose (mg/kg/day)

q* = Cancer potency factor, also known as slope factor (mg/kg/day)

Cd cancer slope factor (CSF) (mg/kg/day) (IRIS,2007) = 6.1 mg/kg-day

Cancer Risk Level

Three range for Lifetime Excess Cancer Risk (LCR) were clearly unacceptable, acceptable and clearly acceptable (USEPA, 2007). Whereby, with 'q' as the risk slope factor and expressed in mg kg^{-1} day^{-1} units. Carcinogenic risk were conservative estimate of the incremental probability that an individual will develop cancer as result of a specific exposure to a carcinogenic compound; the estimation was done by multiplying an individual's lifetime average daily dose (LADD) of a compound by the compound's unit risk factor or cancer slope factor (CSF) (IRIS, 2009).

The acceptable risk was generally defined as 1×10^{-6} for the general public. Cancer potency factor values can be found on EPA's (2009) Integrated Risk Information System (IRIS). EPA guidelines specified that an acceptable risk is a lifetime cancer risk of no greater than 1 in 1,000,000. Then, lifetime cancer risk (LCR) value would be referred to the following table to access the risk acceptability for carcinogenic health effect.

1.6 Study Objectives

1.6.1 General Objective

To determine level of cadmium in canned fish and health risk assessment among students of Faculty of Medicine and Health Sciences, Universiti Putra Malaysia.

1.6.2 Specific Objectives

1) To determine the socio demographic of the respondents.
2) To determine the type and brand of the most favorable canned fish consume.
3) To determine the frequency intake of 2 types of most favorable canned fish by the respondents.
4) To determine the level of cadmium in the 3 types of the most favorable canned fish brand.
5) To compare the cadmium level in 3 types of the most favorable canned fish brand.
6) To compare Average Daily Dose (ADD) and Lifetime Average Daily Dose (LADD) values with Permissible Tolerable Weekly Intake (PTWI) by World Health Organization (WHO).
7) To determine the health risk of respondents indicated by Hazard Quotient (HQ) and Lifetime Excess Cancer Risk (LCR).

8) To determine the relationship between the frequency intake of canned fish and the health risk encountered by respondents.

9) To determine the association between frequency intake of canned fish and respondents health status.

1.7 Hypothesis

1) There are significant relationships between the frequency intake of canned fish and the health risk encountered by respondents.

2) There are significant different between health status of respondents and frequency intake of canned fish.

1.8 Study Limitation

1) It only covers Bachelor Science of Environmental and Occupational Health (KPP) course (year 1-4), Bachelor Science of Community Health Nutrition (PKK) course (year 1-4), and Bachelor Science of Dietetic (BsD) course (year 1-3) and Bachelor Science of Biomedical (BsB) course (year 1-4).

2) There was no biological sample taken in this study.

3) Only two type of the most favorable canned fish was considered in this research.

CHAPTER 2

LITERATURE REVIEW

2.1 Cadmium

Cadmium poisoning was a serious example of the toxicity of some metals in the body. In the body cadmium had no constructive function, meaning it serves no biological function except as a toxin. Cadmium was firstly found by Stroneyer Friedrich on 1817 at Gottingen, Jerman from the pollution of zinc carbonate (Bonnell et al., 1965). Cadmium was highly toxic even at low doses when ingested over a long time period (Ufuk, 2005; Tuzen, 2006). Cadmium can accumulate in our body for a long period of time it is because cadmium half life was in 30 – 40 years. Once taken up by the blood, the majority of cadmium was transported bound to proteins, such as Albumin and Metallothionein (Johannes, 2006). In liver, cadmium will induces the production of Metallothionein. After the cadmium was bind to the protein it was known as Cd-Metallothionein and cadmium excretion was via urine and faeces.

The effects of acute cadmium exposure were flu-like symptoms, fever, chills, muscle aches. These flu-like symptoms were referred to as "The Cadmium Blues". More serious exposure to cadmium had much more detrimental effects. Proximal Renal Tubular Dysfunction occurs when significant amounts of cadmium were ingested, meaning the kidneys lose their ability to remove acid from the blood. Renal tubular damage was known to be irreversible; therefore for diabetic patients especially, they were at high risk of cadmium-induced renal tubular dysfunction (Rubio, 2005).

Cadmium was a widespread non-essential toxic heavy metal, which was known to affect cellular processes through membrane damage, disruption of electron transport, enzyme inhibition or activation and DNA alteration (Smeets et. al., 2005). Although cadmium compounds were relatively poorly absorbed from the gastrointestinal, the occurrence of systemic toxicity following ingestion indicates that the absorption of cadmium from the gastrointestinal tract did occur and therefore that all cadmium compounds should be considered potentially harmful if ingested. Cadmium poisoning will shows symptoms which begin almost immediately after ingestion and include vomiting, diarrhoea and abdominal pain. In severe poisoning, facial oedema, hypertension, pulmonary oedema, metabolic acidosis, oliguria and finally death have been reported. It was also well known that an increased dietary cadmium intake may cause functional disturbance especially in young developing organisms (Rubio, 2005). Other than that, smoking (a cigarette box contains between

2 µg and 4µg Cd), and drinking water were one of the main source of cadmium in human (Rubio, 2005).

2.2 Fish and Heavy Metals in Fish

Fishes were known contain highly nutritious and consumed as a delicacy food throughout country (Prasad and Kumar, 2007; Singh, 2010) because it had high protein content, low saturated fat and also contain omega fatty acids which known to support good health (Ikem & Egeibor, 2005; Tuzen, 2006), moreover fish and canned fish contain micro and macro elements like calcium, phosphorus, fluorine and iodine (Ismail, 2005; Adamczyk, 2008). Other than that, marine fish and product made from them were primary natural source of dietary iodine. They were also rich in microelements, such as selenium, and zinc (Adamczyk, 2008).

Sardine or pilchard, was an ocean going fish well known for travelling in large groups, or schools. The sardine was related to the herring, and sometimes canned fish labelled as sardine was actually herring. The sardine was widely fished in the Pacific and Atlantic, and popular both preserved and fresh in a wide variety of cuisines. Sardines were not considered to be a fish species at risk. It is because careful monitoring of sardine fisheries had been recommended to prevent overfishing and potential collapse.

Sardine was a small, silvery fish with a protruding snout and large mouth. Sardine species can be found in northern and southern waters and favour estuaries or inter-tidal zones. The sardine eats plankton and small fish larva and forms an important part of the marine food chain because they were in turn eaten by larger species. In addition to being eaten by other fish, sardines form a substantial part of the diet of many aquatic birds such as pelicans. The sardine had dark, oily flesh which was popular among some consumers and loathed by others.

As for sardine, it was also rich in omega-3 acids, and generally considered to be very healthy for those who favor the taste. When preserved, sardines were often split and canned with oil after being salted. Sardines were suspended in oil or brine to prevent their oils from going rancid and therefore rarely found dried.

Sardines were canned in many different ways. At the cannery, the fish were washed, their heads were removed, and the fish were cooked, either by deep-frying or by steam-cooking, after which they were dried. They were then packed in olive, sunflower or soybean oil, water, or in a tomato, chili or mustard sauce.

Since, fish was the last link in the aquatic food chain, the heavy metal concentration in many fish species have been determined in relation to the metal content of the aquatic environment (Celik, 2005; Matsushita, 2001). The metals distribution in fish was different depending on the age, species, development status and other physiological factors (Kagi & Schaffer, 1998; Abdollahi, 2004). Metal pollution in marine environment was not very visible but its impact on delicate marine ecosystem and humans were drastic (Ashraf, 2005).

Heavy metals that burdens in fish follow a multivariate dependence pattern. They were not biodegraded and therefore, their bioaccumulation in fish (Anu, 2010) with substantial amount of metals in their tissues especially muscles and thus, represents a major dietary source of these metals to humans (Waqar, 2004; Kalay et al., 1999; Rose, 1999; Tariq et al., 1993; Ashraf et al. 2005; Suhendan, 2010)

2.3 Cadmium in Canned Fish

Levels of heavy metals in fish and canned fish samples have been widely reported in the literature (Ufuk & Oehlenschlager, 2007; Emami et al., 2005; Karadede et al., 2004; Tarley et al., 2001; Voegborlo et al., 1999; Tuzen, 2006). Most of marine fish were canned and this makes it more available for consumption of human that living far away from sea sites and also for individuals that prefer easy life or by those who stretched of time by having this half instant food in their daily dietary.

Canned foods were popular in developing countries and canned fishes were one of the most popular foods (Voegborlo et al., 1999; Ikem and Egeibor, 2005; Lourenco et al., 2004; Ashraf et al., 2006; Khansari et al., 2005; Suhendan, 2010). Fish may be expose to heavy metals in contaminated waters environment during the growth, during canning process (Ikem and Egeibor, 2005; Suhendan, 2010) and also poor handling practices of raw material (Ufuk, 2005; Tuzen, 2006). Furthermore, the concentration of heavy metals in canned fish varies, depending on the type and also origin of the food, the pH of the canned food product, amount of oxygen concentration in the headspace, the quality of the inside lacquer coating of cans, it storage condition and other factors that might affect the canned food (Tahan et al., 1995; Tarley, 2001).

In Finland, fish contributes about 3% of the average Cd intake, over 50% of that cadmium amount was derived from canned, salted or smoked fish (Tahvonen and Kumpulainen, 1996; Rubio. 2005). While from the studies carried out in Malaysia shown that cadmium in canned fish was ranged from 0.07-0.14 μg/g, respectively (Zahari et al., 1987).

However, data on trace heavy metals in canned sardine samples in Malaysia were very limited. Therefore, determination the levels of heavy metals in commercials canned food can provide reliable database for the development of surveillance programs to ensure the safety of the food supply and minimizing humans exposure (Ashraf, 2006; Cid et al., 2001; Dural et al., 2007; Uluozlu et al., 2007; Yilmaz et al., 2007; Suhendan, 2010) and also for the health risk assessment data for future review (Bruce and Bergstrom, 1983; Orejimi, 2000).

2.4 Provisional Tolerable Weekly Intake (PTWI)

PTWI was define as the level of intake of an accumulative contaminant which can be ingested without appreciable health risk over a lifetime intake period by Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee of food Additives (JECFA). WHO also had established PTWI from all sources were 0.007 mg/kg/bw.

2.5 Lifetime Average Daily Dose (LADD) and Average Daily Dose (ADD)

ADD was used for the non-carcinogenic risk which was expressed using the hazard quotient (HQ). For ingestion exposure, HQ is a ratio of the average daily dose (ADD) to the reference dose (RfD). Where,

For ingestion: HQ = ADD (mg/kg/day) / RfD (mg/kg/day)

While, LADD was used for ingestion exposure, the lifetime cancer risk (LCR) was estimated by the product of the lifetime average daily dose (LADD) and the cancer slope factor (CSF). Where,

For ingestion: LCR = LADD (mg/kg/day) x CSF (mg/kg/day)

The acceptability of non-cancer risk was based on the hazard quotient (HQ). A HQ of less than 1 was considered as acceptable as this means that the ADD was below the RfD and the acceptability of cancer risk was 1×10^{-6}.

CHAPTER 3

METHODOLOGY

3.1 Type of Study

This study was a cross-sectional study. This study was to determine the level of cadmium in canned fish and the health risk assessment on students of Faculty of Medicine and Health Sciences, Universiti Putra Malaysia.

3.2 Study Location

The study location of this study was at Faculty of Medicine and Health Sciences, Universiti Putra Malaysia. The reasons why this location was choose because of the awareness effect of heavy metal among the students was high. Other than that, in Malaysia there was no study done to IPTA students and stay in 17 college, UPM.

3.3 Study Population

Table 1: Study Population

COURSE	YEAR 1	YEAR 2	YEAR 3	YEAR 4
Bachelor Science of Environmental and Occupational Health (KPP)	66	40	34	54
Bachelor Science of Community Health Nutrition (PKK)	61	49	48	64
Bachelor Science of Biomedical (BsB)	71	42	46	62
Bachelor Science of Dietetic (BsD)	30	26	25	-

In Table 1 Study Population showed the total number of students for each course following the years of each the courses that will be involved in this study. The total for this study populations were 718 of students in Faculty of Medicine and

Health Sciences, Universiti Putra Malaysia, Serdang, Selangor Darul Ehsan. The populations that have been choosing to involve in this study were students from KPP, PKK, BsB, and BsD courses. For Bs Dietetic students, only students in year 1 till year 3 will be involve and for the other courses, students from year 1 until year 4 will be involve. While for year 4 Bs Dietetic students and Medical students will not be involving in this study. The sample size in this study will be 215 of respondents that will be involved for data collection.

3.4 Data Collection Framework Process

In Figure 3 showed the Data Collection Flowchart of this study. The study proposal must be sent to UPM Ethic Committee to get the approval for this study. The sample size for this study was 215 respondents. The respondents will be sampled by using the random sampling method. The respondents that can participate in this study should follow the criteria set.

Before the respondents can participate in this study, they will be given a consent letter, an explanation on the study objectives, the self-administered questionnaire and before the whole session ends there will be an interview session for interview-administered food frequency questionnaire. There will also be weight and height measurement at the end of the session.

Then, all information in the questionnaires will be analyzed and follows by food sampling analysis. There will be two methods that will be used which were ASH extraction method (Alender, 1970) and Inductively Coupled Plasma - Mass Spectrometry (ICP-MS) method to determine cadmium in the canned food. While the Gannt Chart in Appendix 5 showed the duration of time period for each work process or activities in this study.

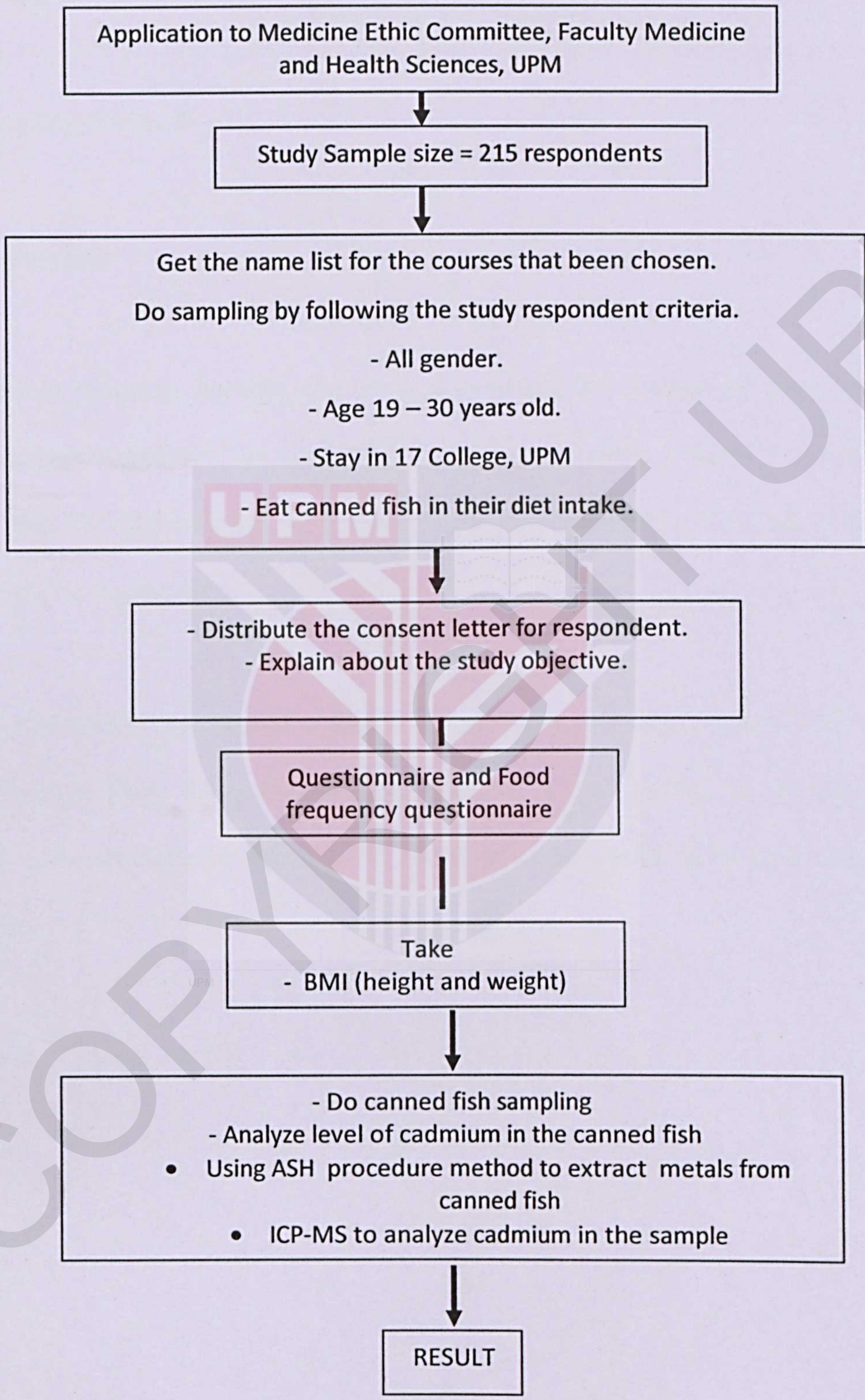


Figure 3: Data Collection Flowchart

3.5 Sampling

3.5.1 Sampling Method

a. Respondent

Random sampling method will be used to select the sample for this study based on inclusive criteria. The samples were randomly selected from 718 study population and 215 respondents were selected for data collection to complete this research (Refer to Figure 4).

For health risk assessment, calculation of Average Daily Dose (ADD) or Lifetime Average Daily Dose (LADD) and Hazard Quotient (HQ) or estimation cancer risk. The respondents also will be involved in measuring their Body Mass Index (BMI).

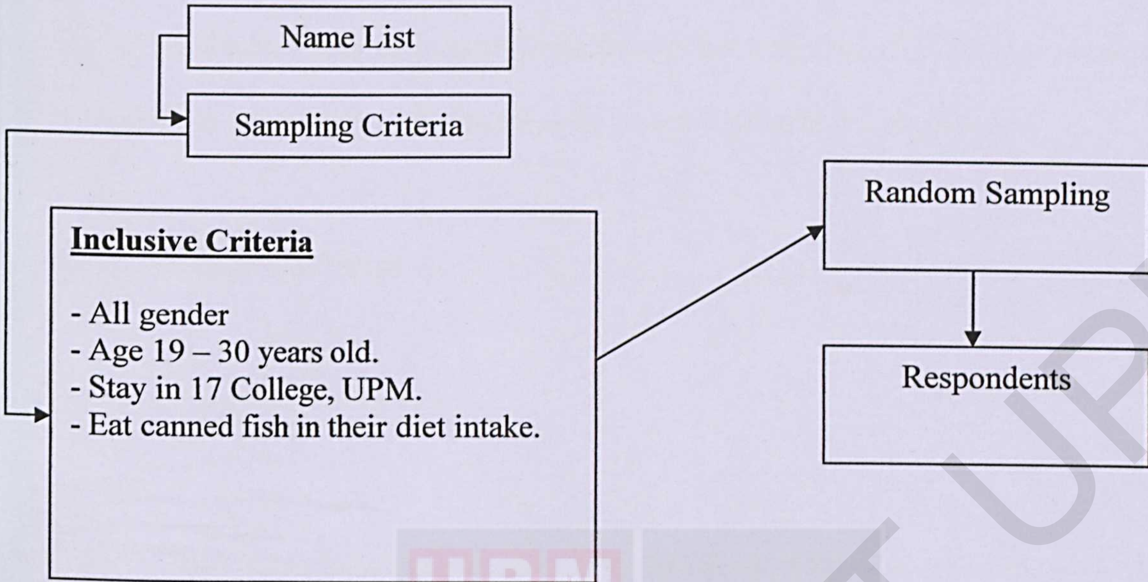


Figure 4: Sampling Method of Respondent

3.5.2 Sample Size

Sampling ratio x size of the target population.

For small population (< 1000) sampling ratios of up to 30% may be required for a high degree of accuracy (Blaikie, 2010)

(30/100) x 718 = 215 respondents

As for the precaution step in this study, data collection that will be taken should not be less than 215 respondents but can be more than 215 data collection of respondents to ensure the sample size was representative to the population.

3.5.3 Sampling Method

a. Food

The food sampling method will be done after the screening of the questionnaire and also the food frequency questionnaire. The highest brand and type of canned fish choose by the respondents will be taken as the sample. The sample was the brand and type of canned sardine that was the most favorable among the respondents. This sample was then will be purchase at the local markets which also had the highest preferable place to the respondents. Food samples will be bought from there. After that, these samples will be put in the refrigerator at -20°C before the analysis as shown in Figure 5.

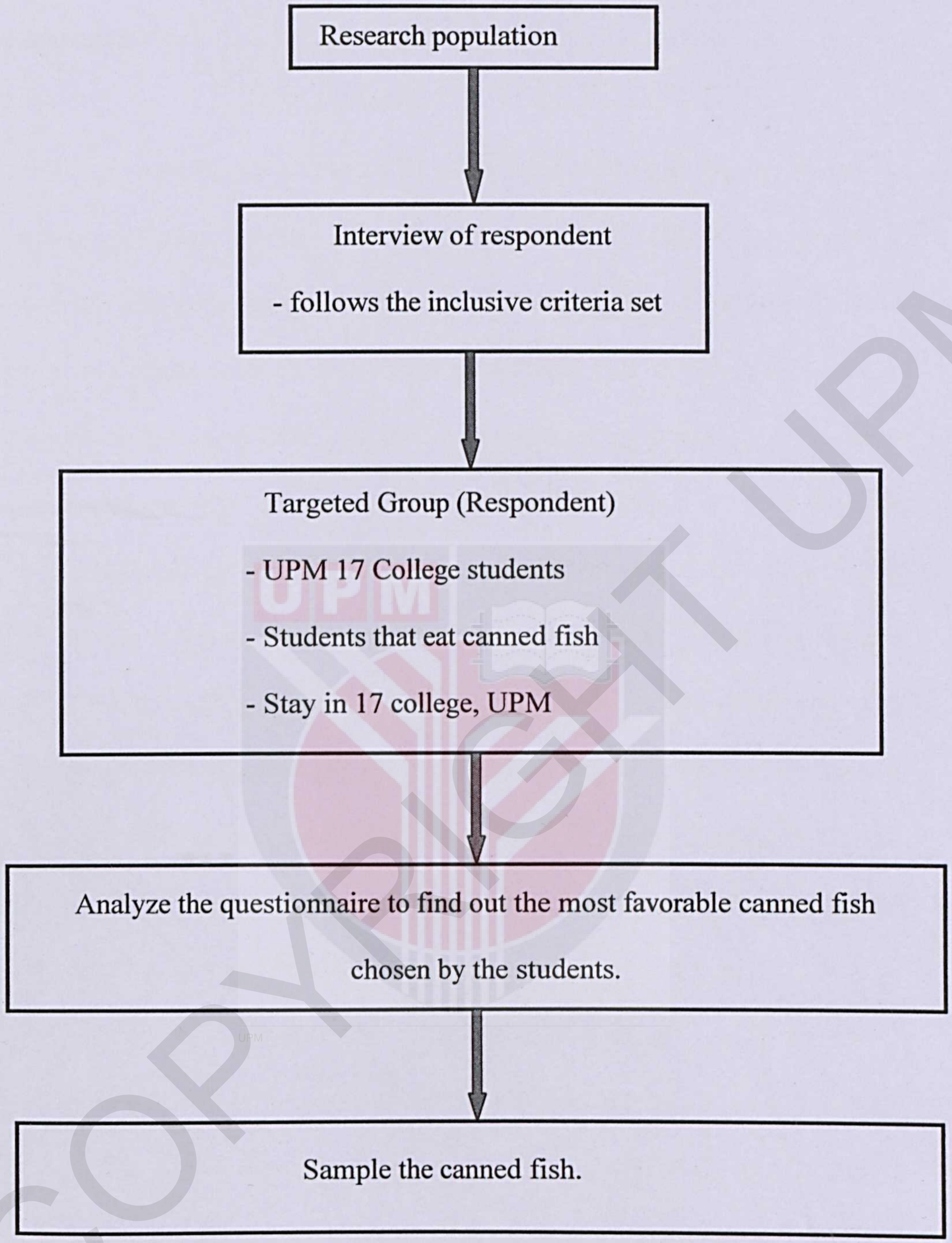


Figure 5: Workflow for canned fish sample

3.6 Instrumentation

Cadmium in sample was extracted by using ASH method and then analyze by using Inductively Coupled Plasma - Mass Spectrometry (ICP-MS) (refer to Figure 6 and 7 above) with unit ppm and change to µg/L. The principle of Inductive Coupled Plasma (ICP) was where atom or ions of the element present in the sample absorb energy, causing excitation of their electrons to unstable energy states. When these atoms or ions return to their stable ground-state configuration, they emit light of specific wavelengths characteristic of the elements. ICP was an argon plasma that can heat the sample to a very high temperature in the range 5500 to 8000°C. Sample was injected as an aerosol, and transported onto the ICP by argon, which was also used as a carrier gas. The emitted light was separated according to its wavelength and measures. For ICP-MS it was method combine an ICP with a quadrupole mass spectrometer to separate the ions produced. High energy ICP generates single charged ions from the atoms of the elements present in the sample. Such ions were now directed onto the mass spectrometer, separated, and measured according to their mass-to-charge ratio. ICP-MS was also known as an analytical technique that performs elemental analysis with excellent sensitivity. The typical detection limit for ICP-MS was 0.01-1 microgram per liter in solution. ICP-MS can perform very fast whereby it can detect up to 60 elements in a 2 minutes scan. Last but not least, Table 2 below showed the standard daily performance of ICP-MS before starting the sample analysis.

Figure 6: Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)

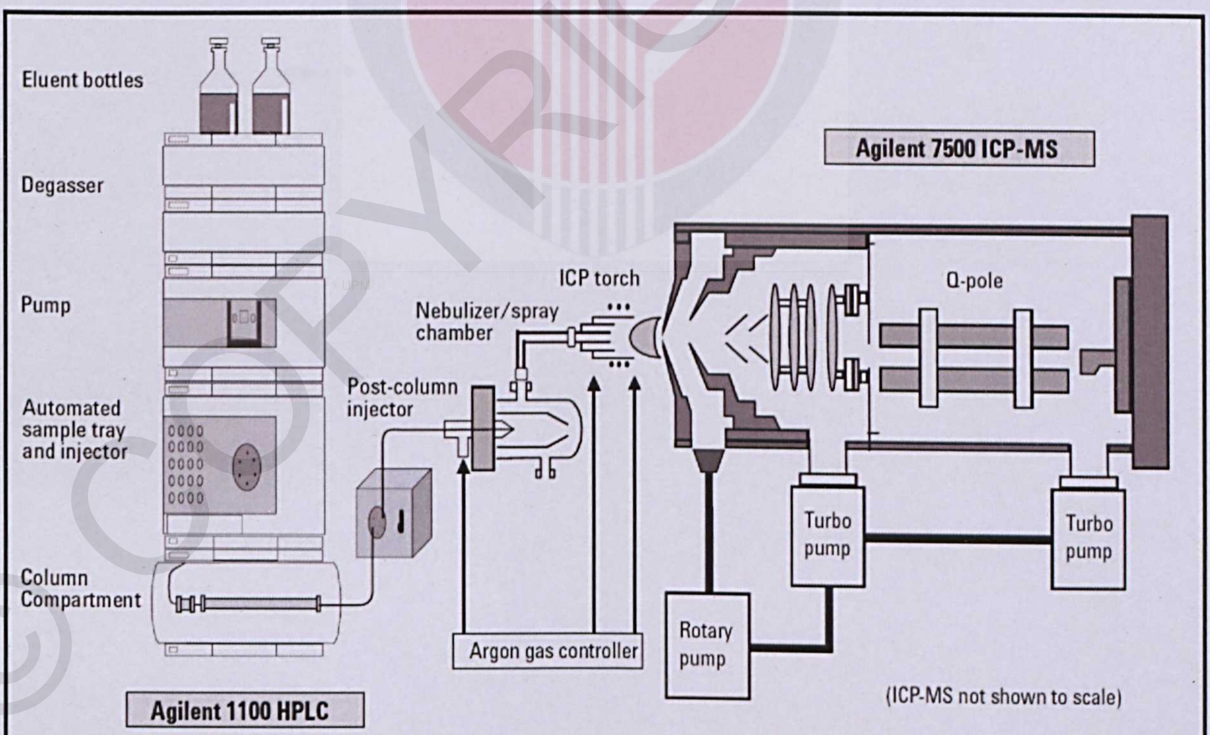


Figure 7: ICP-MS Coupling System

Table 2: Standard Daily Performance of ICP-MS

Current Value	Description
0.72	Nebulizer Gas Flow [NEB]
1.20	Auxiliary Gas Flow
17.00	Plasma Gas Flow
8.00	Lens Voltage
1100.00	ICP RF Power
-1700.00	Analog Stage Voltage
750.00	Pulse Stage Voltage
0.00	Quadrupole Rod Offset Std. [QRO]
-12.00	Cell Rod Offset Std. [CRO]
25.00	Discriminator Threshold
-26.00	Cell Path Voltage Std. [CPV]
0.00	RPa
0.25	RPq
0.91	DRC Mode NEB
-5.50	DRC Mode QRO
-0.50	DRC Mode CRO
-16.00	DRC Mode CPV
0.00	Cell Gas A

Muffle Furnace (Figure 8) were designed for laboratory and industrial applications. It was used for engineering tests of soils and aggregates, cement testing, ashing organic and inorganic samples, gravimetric analysis, ignition tests and others. The furnace was also insulated with Insu-Light molded ceramic fiber insulation a percentage input controller allows desired temperature to be selected with repeatable results. It also features a fluorescent digital display. While in Figure 9 shown was the crucible which was used to place the food sample into it before starting the dry ashing in the muffle furnace.

Figure 8: Muffle Furnace

Figure 9: Crucible in the Muffle Furnace

3.7 Data Collection

3.7.1 Questionnaire

All questionnaires were distributed to the respondent to finds out their personal details and others information. Other than that, the objectives of this study were explained to the respondents as in the respondent information sheet (Appendix 2).

For data collection, in this study there were two type of questionnaire. The first one was self-administered questionnaire (Appendix 4). It had 6 parts overall which were respondents details, health status, Body Mass Index (BMI), cadmium exposure from food, Food Frequency Questionnaire (FFQ) and other sources that can contribute to cadmium exposure.

3.7.1.1 Part 1 - Respondent details

In Part 1 was about getting respondent personal details. Details that were needed were full name, name of course, age, gender, weight, height, race, religion, level of education, financial source, monthly expenses, food expenses only, and period have been stay in 17 College, UPM.

3.7.1.2 Part 2 - Health Status

In Part 2 was about respondent health status either they have disease that might cause from cadmium poisoning such as kidney failure or disease that can highly affected to cadmium poisoning such as diabetes and also symptoms such as stomached, diarrhea and others.

3.7.1.3 Part 3 - Body Mass Index (BMI)

In this part Body Mass Index of the respondent was calculated from the information of height and weight of the respondent. Where,

$$\text{BMI} = \quad \text{Weight (kg) / Height x Height (m}^2\text{)}$$

3.7.1.4 Part 4 - Cadmium exposure from food

While in Part 4 was about information on where respondent usually eat, what type of brand and canned fish they usually eat and place where respondent usually get their source of canned fish.

3.7.1.5 Part 5 - Food Frequency Questionnaire (FFQ)

The FFQ in this study was use to assess the respondents intake of various type of food. In FFQ contains few types of food categories such as carbohydrate, protein, vegetables, seasoning and process food. There were also 7 frequency intake (everyday, once per week, 2-6 times a week, once per month, 2-3 times a month, seldom, do not consume) and there was also column for amount of quantity food take (Appendix I). Respondents were asked to report all their food frequency intakes for each type of food listed in the questionnaire. The FFQ was modified from Malaysian Adult Nutrition Survey, 2002-2003.

Respondents were also asked to answer or state the frequency for each type of food listed being taken in the FFQ. Explanation was given to the respondents before answering the questionnaires. Interview session was held when respondents did not understand or did not answer the self-administered questionnaire precisely or correctly and for the FFQ it was filled up by the researcher during the interview session. This was to make sure that all the answers given were correct.

3.7.1.6 Part 6 - Other sources that can contribute to cadmium exposure

Last but not least, Part 6 the last part of the questionnaire was about getting the information on other sources that can contribute to cadmium exposure to respondent were source of drinking water, smoking habit and alcohol intake.

3.7.2 Lifetime Average Daily Dose (LADD) and Average Daily Dose (ADD)

While, for the health risk assessment, average daily dose (ADD) or lifetime average daily dose (LADD) will be calculate to determine the intake of cadmium in food by using the following formula:

$$ADD / LADD = C \times IR \times ED \times EF / BW \times AT$$

Whereby;

ADD / LADD – Intake of cadmium (mg/kg/day)

C – Total of cadmium concentration in canned fish (mg/kg)

IR – Ingestion rate of canned fish (kg/day)

ED – Exposure duration (year)

EF – Exposure frequency (day/year) [EF = (F (day/year) x ED) / AT]

BW – Body weight (kg)

AT – Averaging time (days)

[ED x 365 day-year] (For ADD non- carcinogenic contaminants)

- Life time equal to 25,550 days based on lifetime exposure for 70 years (LADD for carcinogenic contaminants)

Estimation of risk for non-carcinogens

$$\text{Hazard Quotient (HQ)} = \frac{\text{ADD}}{\text{Oral RfD}}$$

Where:

Hazard Quotient = Non-cancer Hazard Index of a health effect from intake of cadmium

ADD = Average daily dose (mg kg^{-1} day^{-1})

Oral RfD = Oral Reference dose of cadmium (mg/ kg/day)

RfD Cd – 5.0×10^{-4} μg per kg body weight (Nielsen, 1996)

Estimation of risk for carcinogen

Lifetime Excess Cancer Risk (LCR) = LADD x q*

Where:

LCR = Upper limit cancer risk

LADD = Lifetime average daily dose (mg/kg-day)

q* = Cancer potency factor, also known as slope factor ($mg\ kg^{-1} day^{-1}$)

Cd cancer slope factor (CSF) ($mg\ kg^{-1}\ day^{-1}$) = 6.1

Cancer Risk Level

A HQ of less than 1 was considered as acceptable as this means that the ADD was below the RfD. Figure 10 showed the range for Lifetime Excess Cancer Risk. For the one in million or 1×10^{-6} was acceptable risk level for a potential fatal event such as cancer.

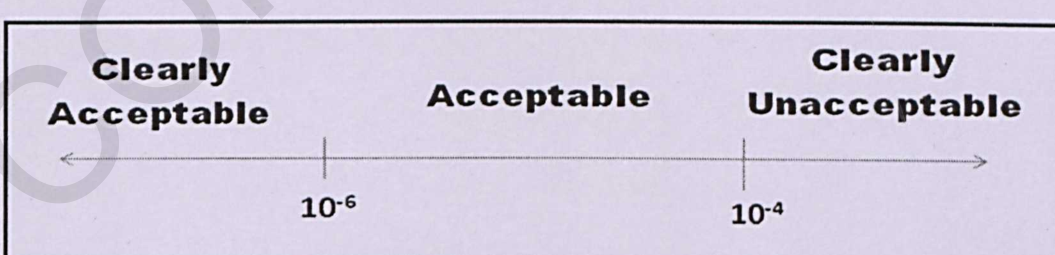


Figure 10: Lifetime Excess Cancer Risk

3.7.3 Samples of Canned Fish

Canned fish samples that being keep in refrigerator was taken out to cool it down under room temperature before weight it for digestion process. Apparatus that was used in this sampling process were digital sample weight machine, spatula, beaker, parafilm, crucible, test tube and stationary. Each sample will be sampled 10 g accurately into the crucible by using the digital weighing machine and each crucible will be labeled and covered with parafilm to avoid sample contamination.

3.8 Food Sample Analysis

The extraction method was done through ASH method. The ash of a foodstuff was the inorganic residue remaining after the organic matter had been burnt away. Hence ash content can be determined by incinerating a known quantity of a foodstuff, previously dried, until constant weight was obtained. ASH method should not be done at temperature exceeding 650□C, at which temperatures inorganic salts like alkali chlorides will volatilize. Moreover, a portion of the ash will fuse and enclose some carbon, preventing them from being ignited. Then, follow with the digestion of the food samples that referred from Zeng-Yei Hseu (2004). The digestion and ASH method of food samples was shown below in Figure 11.

10 g of each sample was weight using the digital weighing machine in the crucible. The sample was placed in the furnace and the muffle furnace was preheated at 200-250°C for 30 minutes and then ash it for 4 hours at 480°C.

↓

Remove sample from the furnace. Cooled it down, add 20 ml of 5 Mol HNO_3 and evaporate it to dryness in desiccators for overnight.

↓

Place sample in a cool furnace and heat it for 400°C for 15 minutes, and before removed (from furnace, cooled it and moist it with 40 drops of distilled water)

↓

Add 20ml of Conc. HCl and evaporate the sample to dryness, remove and add 50ml of 2 Mol of HCl and swirled the conical flask.

↓

Filtered the solution through Whatman No.42 filter paper and less 0.45micrometer milipore filter.

↓

Transfers quantitatively to 250 ml volumetric flask by adding diionize water.

Figure 11: Dry ashing workflow

In some food it was difficult to burn away all the carbon. The ashing process can be assisted by treating it with hot distilled water. Then we should prepare the ash solution for the determination of cadmium and 20mL of concentrated hydrochloric acid was added to the ash with rinsing of upper portions of the dish. Then let it evaporate to dryness over water-bath. Last but not least, 50mL of 2Mol concentrated hydrochloric acid will be added and the solution will be filtered by using Whatman No. 42 and less than 0.45 micrometer milipore filter into the conical flask. The solution will then be transferred quantitatively into 250ml volumetric flask by adding deionize water and before running the sample in ICP-MS, all steps and procedure in Figure 12 was followed;

Make the standard solution for ICP-MS by using the concentrated ICP-MS standards solution given. Make the standard solution into 10ppb, 30ppb, 50ppb and 100ppb by using $M_1V_1 = M_2V_2$ formula.

⬇

Pour the deionize water into the blank plastic tube, then follow with the standard solution (10ppb, 30ppb, 50ppb and 100ppb) and the extracted sample solution into the plastic tube (duplicate each sample solution) given in zigzag arrangement on the rack provided.

⬇

Run all the samples by using ICP-MS (Before that, ICP-MS must be run first to ensure it reach the standard level of condition before able to run any samples)

⬇

All results were printed out.

Figure 12: Steps and procedures to run ICP-MS

3.9 Calculation for Cadmium Concentration from ICP-MS result

Concentration of sample (mg/kg) = (AxB) x C/W

Where,

A = the concentration of metal in the extract/ from ICP-MS result (μg/mL)

B = dilution Factor (4)

C = the volume of extract (60 mL)

W = weight of sample (10 g)

Example sample 1:

A = 0.806 μg/L = 0.000806 μg/mL

B = 4

C = 60 mL

W = 10 g

Calculation:

Cd mg/kg = (0.000806 μg/mL x 4) x 60mL / 10 g

= 0.019344 μg/g

= 0.019344 mg/kg

3.10 Quality Control

Pre-test was done to 10% equals to 25 students from the sample size which had the same population but at different place. This was to increase the quality of the questionnaires for this study. For the instrumentation, the sample will be analyzed by using ICP-MS and all samples were duplicated to ensure the precision of the test result. While for the interview session, practice on interviewing respondents will be done by the researcher to make sure the researcher was used with the questions that will be asked by the respondents.

3.11 Data Analysis

a. Univariate analysis

Data collected from this study were then analyzed by using SPSS (Statistical Package for Social Science) version 19.0 and Microsoft Excel. This was to get min, median, mode and standard deviation study data. All the data will be getting from the questionnaire. The descriptive analysis will be done for data such as age, education level and concentration of cadmium in canned fish and the frequency of consuming.

b. Bivariate analysis

Descriptive analysis and bivariate statistical analysis such as Chi square test and Spearman's rho Correlation test was used to determine the association between frequency intake and health risk encountered by respondent with the probability of error set at $p < 0.05$.

3.12 Ethical Approval

Ethical approvals for this study were obtained from Medicine Research Ethic Committee of Faculty Medicine and Health Science, UPM prior to the study (Appendix 1). Other than that, respondent consent form (Appendix 3) must be returned to researcher to ensure that respondents were agree to participate in the research. All details were confidential and not for public review. Result for this research will be informed through phone contact or meeting with the researcher.

CHAPTER 4

RESULT

This research had been conducted among 17^{th} College students in Universiti Putra Malaysia from 21 January 2012 to 18 March 2012. Four courses were involved in this research. Respondent who involved in this research were only students who ate canned fish and stayed in Kolej 17, UPM.

Table 4.1.1, 4.1.2, 4.2.1, 4.3.1, 4.4.1, 4.4.2 and 4.5.1 shows the descriptive data of this research. All these information was from the questionnaire where it have 5 parts which were part 1 respondents details, part 2 respondents health status, part 3 respondents Body Mass Index, part 4 respondents exposure to cadmium through food intake, part 5 Food Frequency Questionnaire, and last but not least part 6 was about other exposure factors that can contribute to cadmium poisoning. While the polar data was cadmium concentration (mg/kg) and non-polar data was the health risk assessment of respondents.

4.1 Socio-demographic of respondents.

Table 4.1.1 presents the socio-demographic of respondents. Majority of the respondent were female which was 152 (71%) from the total respondents. There were four types of races among the respondents which were Malay, Chinese, Indian and others. More than half of the respondents were Malay ethnicity (76%) and the respondents were also consisting mainly from Islam religion (77%).

In this study, the total number of respondents from PKK course had the highest number of involvement in this research with 64 people. Respondents from KPP were the second highest respondents involvement with 62 people. More than half (75%) of the respondents were obtained education until matriculation or pre-university. Other than that, out of the 215 respondents, 51% of them received their financial source from JPA, follow by PTPTN with 39%, 6% from parents and another 6% from other organization that provide financial source for students such as Yayasan Tunku Abdul Rahman.

In this study, the age of respondents that involved were range from 19 to 30 years old with majority of the respondents were in range in 22 to 26 years old while the range for height of respondents was 1.5 to 1.8 meter tall as in Table 2. Body Mass Index (BMI) result of respondents showed four weight grades which underweight, normal, overweight and obesity. Majority of the respondents weight grade were at normal grade (65%) and only 4% of the respondents were in obesity grade. Furthermore, majority of the respondents spends half of their money only for food expenses purposes with respectively RM270 from RM400. The minimum monthly expenses among respondents were RM100 and maximum were RM1200. Meanwhile, the expenditure only for food purposes was RM50 for the minimum expenses and maximum was RM500 a month.

Table 4.1.1: Background Information of respondents (N=215)

Variables	Frequency	Percentage (%)
Gender		
Male	63	29
Female	152	71
Race		
Malay	164	76
Chinese	38	18
Indian	5	2
Others	8	4
Religion		
Islam	166	77
Christian	13	6
Buddhist	32	15
Hindu	4	2
Courses		
Bs. KPP	62	29
Bs. PKK	64	30
Bs. Biomedical	60	28
Bs. Dietetic	29	14
Education Level		
STPM	60	14
Matriculation/Pre-University	162	75
Diploma	23	11
Financial Source		
JPA	109	51
PTPTN	83	39
Parents	10	5
Others	13	6

Table 4.1.2: Background Information of respondents (N=215) (Continue)

Variables	Frequency (%)	Median (IQR)	Range
Age (Years)			
19-21	100 (46)	22 ± 2	19 - 30
22-26	111(52)		
27-30	4 (2)		
Height (Meters)		1.6 ± 0.1	1.5 - 1.8
Weight (Kilogram)		52 ± 12	36 - 105
BMI			
Underweight (< 18.4 kg/m^2)	50 (23)		
Normal (18.5–24.9 kg/m^2)	140 (65)	20 ± 4	14 -36
Overweight (25.0–29.9 kg/m^2)	17 (8)		
Obesity (≥ 30.0 kg/m^2)	8 (4)		
Monthly Expenses (RM)		400 ± 200	100 - 1200
Food Expenses (only) (RM)		270 ± 100	50 - 500

4.2 Health information of respondents

The information regarding health information of respondents gathered was showed in Table 4.2.1. From the result it showed that only 3 respondents having a disease from the total number of respondents and majority 212 respondents do not have any disease. While for the symptoms, majority of the respondents were having headache (13%) and follow by 10% of the respondents were having stomach ache. Nevertheless, only small numbers of respondents were having vomiting, diarrhea, muscle tense and difficulties to breathe.

Table 4.2.1: The prevalence of possible disease and symptoms of cadmium poisoning among respondents (N=215)

Variable	Frequency (%)	
	Yes	No
Disease		
High Blood Pressure	3 (1)	212 (99)
Symptoms		
Stomach ache	22 (10)	193 (90)
Vomiting	4 (2)	211 (98)
Diarrhea	4 (2)	211 (98)
Muscle tense	4 (2)	211 (98)
Headache	27 (13)	188 (87)
Difficulties to breath	3 (1)	212 (99)
Lethargy	17 (8)	197 (92)

4.3 Information of Canned Fish Choose by Respondents

Table 4.3.1 and Table 4.3.2 showed information of canned fish chooses by respondents. Majority 72% of the respondents consumed sardine canned fish, while for the less favorable type of canned fish were tuna hot mayonnaise, and tuna in water which had only 1 people consume each type of those canned fish. A total from 215 people of respondents, 107 people of the respondents had chosen to eat in their hostel. While for the most favorable brand of canned fish consume by the respondents was Ayam Brand which 83% from the total amount of the respondents and followed with King Cup as the second most favorable type of canned fish consumed by the respondents. Last but not least, place where majority of respondents bought canned fish was Giant Supermarket, The Mines with 54% of people.

Table 4.3.1: Information of canned fish choose by respondents (N= 215)

Variables	Frequency	Percentage (%)
Type of Canned Fish		
Sardine	152	71
Tuna	32	15
Mackerel	10	5
Tuna Spread	11	5
Tuna Mayonnaise	5	2
Tuna Chili	3	1
Tuna Hot Mayonnaise	1	0.5
Tuna in Water	1	0.5
Location Eating Canned Fish		
K 17 cafeteria	24	11
Serdang	52	24
K 17 Hostel	107	50
Others	32	15
Brand of Canned Fish		
Ayam Brand	179	83
King Cup	16	7
Sardine	6	3
Roda	2	1
Adabi	3	1
Marina	4	2
Cinta	1	1
Others	4	2

Table 4.3.2: Information of canned fish choose by respondents (N= 215) (Cont.)

Variables	Frequency	Percentage (%)
Location Bought Canned Fish		
K17 Shop	42	20
Mines Giant	117	54
Big Hypermarket	25	12
Tesco	11	5
Jusco	7	3
Mydin	1	1
Econsave	3	1
Others	9	4

4.4 Food Frequency Intake of Respondents

Figure 13 showed the frequency intake of two most favorable canned fish by respondents which were sardine and tuna spread. Only 3% of respondents consumed sardine canned fish every day. While for tuna spread canned fish, 27% of respondents consumed it every day. For once a week frequency intake, sardines and canned tuna canned fish consumed respectively 16% and 9% of respondents and for once a month 25% of respondents consumed sardine and 27% of respondents consumed tuna spread. Last but not least, 22% of respondents seldom consumed sardine and 9% of respondents seldom consumed tuna spread.

Table 4.4.1 showed the result on frequency intake of top 10 most consumed foods by respondents. The highest food intake was rice which 201 people. Then followed by leafy vegetables with 116 people consumed it. Meanwhile, a cereal was the third most consumed food by the respondents with 36 people. Other than that, numbers of respondents that consumed food additives shrimp, dark soya sauce and oyster sauce with respectively 27 people, 25 people and 15 people.

Furthermore, result gathered in Table 4.4.2 was frequency intake of top 10 weekly consumed foods by respondents. Majority of the respondents consumed marine fish (57%). While bitter, pumpkin, or cucumber had the second most weekly consumed by respondent (41%). 39% of respondents consumed root vegetables, 36% of respondents consumed anchovies and leafy vegetables was consumed by 32% of respondents. There were few more other foods consume weekly as in the table presents.

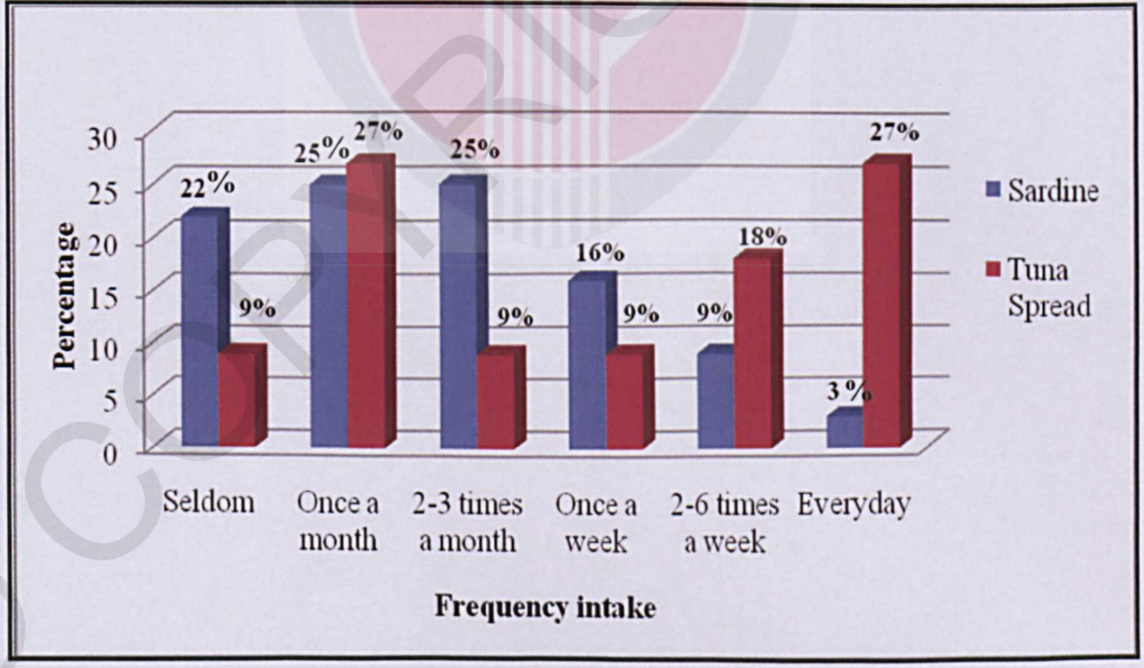


Figure 13: Frequency intake of 2 most favorable canned fish by respondents

Table 4.4.1: Frequency of top 10 most consumed foods by respondents

Type of food	N	%	Mean frequency per day	Total amount consume a daily
Rice	201	94	2	1 plate
Cereals	36	17	0.2	1 Chinese bowl
Marine Fish	24	11	0.1	1 tail
Anchovies	22	10	0.1	1 tablespoon
Leafy vegetables	116	54	1	1 cup
Root vegetables	19	9	0.1	1 cup
Bitter/pumpkin/cucumber	20	9	0.1	1 cup
Shrimp	27	13	0.2	1 tablespoon
Dark soya sauce	25	12	0.2	1 teaspoon
Oyster sauce	15	7	0.1	1 tablespoon

Table 4.4.2: Frequency of top 10 weekly consumed foods by respondents

Type of food	N	%	Mean frequency per day	Total amount consume daily
Marine fish	122	57	2	1 tail
Anchovies	77	36	1	1 tablespoon
Prawn	47	22	0	3 tail
Fish balls/fish cake	62	29	1	3 balls/ pieces
Fish crackers	49	23	0	4 pieces
Leafy vegetable	69	32	6	1 cup
Root vegetable	84	39	1	1 cup
Bitter/pumpkin/cucumber	88	41	2	1 cup
Shrimp	54	25	2	1 tablespoon
Dark Soya Sauce	59	27	2	1 teaspoon

4.5 Other Possible Sources of Cadmium Exposure to Human

Besides that, other possible sources of cadmium exposures to human were gathered in Table 6. Majority 81% of the respondents choose their source of drinking water was from tap water and 19% choose other sources of drinking water such as water cooler and mineral water. Meanwhile, 7% of respondents consumed alcohol. The results also showed the intake and duration of smoking. Only 3% from the total number of respondents were smoking. The range number intake of cigarettes was 0 to 20 sticks a day and among the total number of respondents, there was an individual that had been smoking for about 33 years long.

Table 4.5.1: Other possible sources of cadmium exposure to human (N = 215)

Variables	Frequency	Percentage (%)	Mean ± SD	Range
Sources of drinking water				
Tap water	174	81		
Others	41	19		
Smoking				
Yes	6	3		
No	209	97		
Alcohol consumption				
Yes	14	7		
No	201	94		
No. of cigarette (stick)			0.3 ± 2	0 - 20
Years of smoking			0.4 ± 3	0 - 33

4.6 Cadmium Concentration in Canned Fish

Table 4.6.1 showed the Parameter used in equation A to convert raw data of ICP-MS to actual reading of Cd concentration. Where by equation A was;

$$\text{Cd conc. (mg/kg)} = (A \times B) \times \frac{C}{W}$$

With,

A= concentration of arsenicin the extract (µg/ml), B=Dilution factor, C= the volume of extract (ml), W= weight of sample (g).

Table 4.6.1: Parameter used in equation A to convert raw data of ICP-MS to actual reading of Cd concentration.

Parameter	Symbol	Unit	Parameter characteristic				
			S1*(M)	S1*(W)	S2*(M)	S2*(W)	S3*(TS)
Concentration of cadmium in the extract	A	µg/mL	8.06x10-4	7.95x10-4	6.48x10-4	6.87x10-4	0.96x10-4
Dilution factor	B	-	4	4	4	4	4
Volume of extract	C	mL	60	60	60	60	60
Weight of sample	W	g	10	10	10	10	10

S*=Sample

Table 4.6.2 showed concentration of cadmium in 3 types of canned fish that were the most consumed by the respondents. From the result, mean concentration of cadmium from concentration 1 and concentration 2 in fish muscle for Ayam Brand Sardine canned fish (0.0192 mg/kg) was higher than King Cup Sardine canned fish (0.0161 mg/kg). While, for cadmium concentration in fish water for Ayam Brand Sardine canned fish (0.0090 mg/kg) was lower than cadmium concentration in King Cup Sardine canned fish (0.0179 mg/kg). The overall result showed, the mean concentration of cadmium in King Cup Sardine canned fish was 0.0029 mg/kg higher than mean concentration of cadmium in Ayam Brand Sardine canned fish. Meanwhile, Ayam Brand Tuna Spread canned fish had the lowest concentration of cadmium from all which only 0.0025 mg/kg. With this, the average total of cadmium concentration level in 3 types of canned fish samples were 0.0130 mg/kg.

Table 4.6.2: Concentration of cadmium in 3 types of canned fish

Canned Fish	Conc. 1 (mg/kg)	Conc. 2 (mg/kg)	Mean Conc. (mg/kg)
Ayam Brand Sardine			
Fish muscle	0.0193	0.0191	0.0192
Fish water	0.0097	0.0083	0.0090
King Cup Sardine			
Fish muscle	0.0156	0.0165	0.0161
Fish water	0.0180	0.0177	0.0179
Ayam Brand Tuna Spread	0.0023	0.0026	0.0026
Average mean Cd. conc.			**0.0130**

4.7 Health Risk Assessment from Consumption of Canned Fish

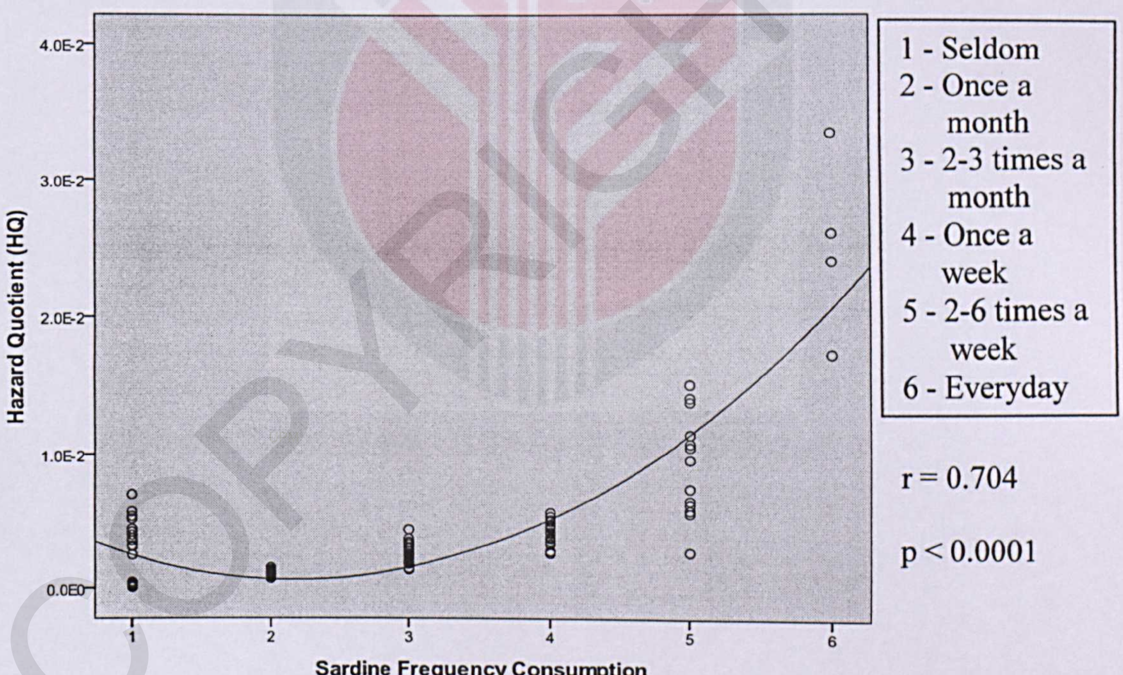
Table 4.7.1 showed the results gathered in Table 8 showed result on Lifetime Average Daily Dose (LADD) and Average Daily Dose (ADD) of respondents. Overall result showed, the mean value of ADD was $2.357x10^{-6} \pm 3.8206x10^{-6}$ mg/kg/day and $3.437x10^{-7} \pm 6.217x10^{-7}$ mg/kg/day was the mean value for LADD. Other than that, the table present the result on the estimation of risk for non-carcinogen; Hazard Quotient (HQ) and estimation risk for carcinogen; Lifetime Excess Cancer Risk (LCR) of respondents. From the result obtained, it showed that the HQ for all respondents was less than 1 and it was considered as acceptable. While for Lifetime Excess Cancer Risk (LCR) also showed none of the respondents had LCR higher than $1 x10^{-4}$. All LCR values were in range between $1 x 10^{-6}$ to $1x10^{-4}$ which in an acceptable range.

Table 4.7.1: Health risk assessment of respondents indicated by the ADD, LADD, HQ and LCR (N=215)

Variable		N	%	Median(IQR)	Mean ± SD	Range
ADD (mg/kg/day)					$2.357 \times 10^{-6} \pm 3.8206 \times 10^{-6}$	
LADD (mg/kg/day)					$3.437 \times 10^{-7} \pm 6.217 \times 10^{-7}$	
HQ						
<1	Acceptable	215	100	0.0234×10^{-5} (0.0338×10^{-5})		$0.0047 \times 10^{-5} – 6.17 \times 10^{-5}$
>1	Not Acceptable	0	0			
LCR						
< 10ˉ6	Clearly Acceptable	0	0			
10^{-6} - 10^{-4}	Acceptable	109	51	981.0×10^{-5} (16.1×10^{-5})		$3.1 \times 10^{-5} – 100 \times 10^{-5}$
>10^{-4}	Clearly not acceptable	0	0			

4.8 The Relationship between the Frequency Intake of Canned Fish and the Health Risk Encountered by Respondents

Figure 13 and Figure 14 presents the result of statistical analysis using the nonparametric spearman's rho test. It showed that there were significant relationships between both frequency intake of sardine and tuna spread canned fish with the health risk encounter by respondents. The p values for both LCR and HQ of sardine were less than 0.001 and p values for LCR of tuna spread was less than 0.05 while HQ less than 0.001.



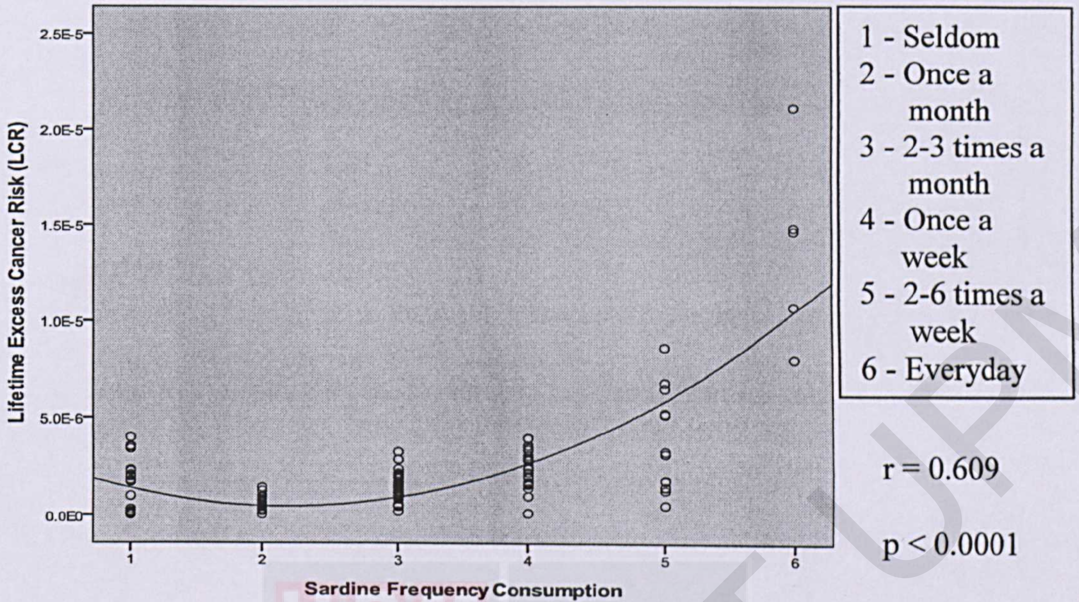
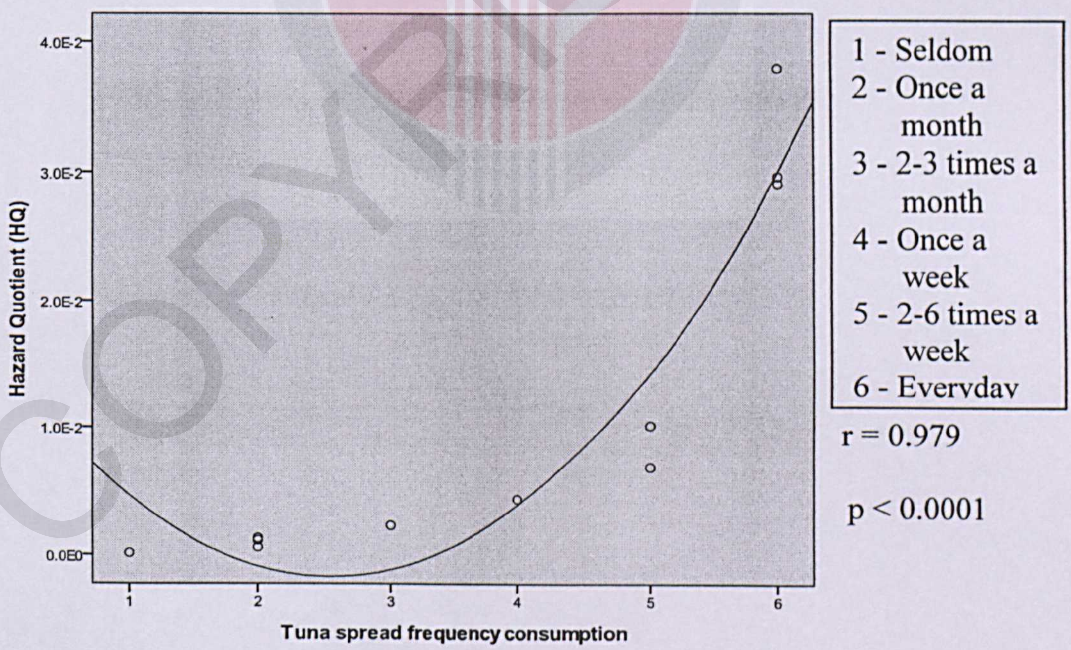


Figure 14: Relationship between the frequency intake of canned fish and health risk encountered by respondents (Sardine)



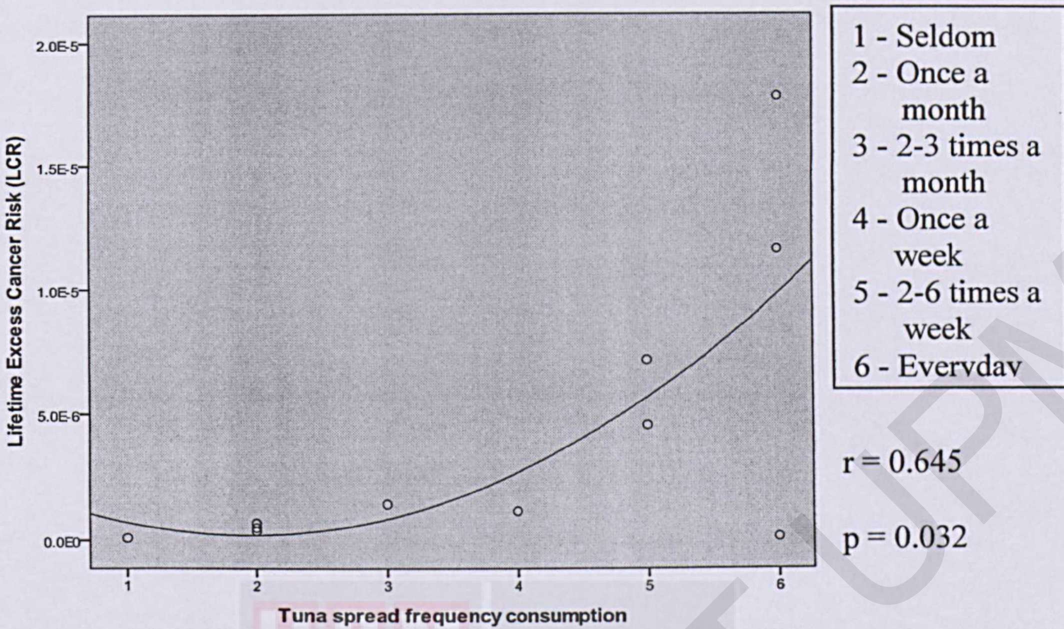


Figure 15: Relationship between the frequency intake of canned fish and health risk encountered by respondents (Tuna Spread)

4.9 The Association between Frequency Intake of Canned Fish and Respondents Health Status

Besides that, the association between frequency intake of canned fish and respondents health status were determine from chi-square test. The result showed there were significant association between frequency intake for sardine canned fish and respondents health status which was symptom of diarrhea. While for tuna spread canned fish showed no significant result.

Table 4.9.1: Association between frequency intake of sardine canned fish and respondents health status

Variable		The Frequency Intake Of Sardine		$_aX^2$	p value
		Frequent	Not Frequent		
Disease					
High Blood	Yes	0	3	0.0001	1.000
Pressure	No	12	137		
Symptoms					
Stomach ache	Yes	2	15	0.101	0.750
	No	10	127		
Vomiting	Yes	1	0	0.324	0.569
	No	11	138		
Diarrhea	Yes	2	1	7.462	**0.006***
	No	10	139		
Muscle tense	Yes	1	1	0.815	0.367
	No	11	139		
Headache	Yes	1	16	0.0001	1.000
	No	11	124		
Difficulties to	Yes	0	3	0.0001	1.000
breath	No	12	137		
Lethargy	Yes	1	12	0.0001	1.000
	No	11	128		

* Continuity Correction **N=152**
2 cell (50%) have expected count less than 5.
$_a$Chi-Square Test

Table 4.9.2: Association between frequency intake of tuna spread canned fish and respondents health status

Variable		The Frequency Intake Of Tuna Spread		$_bX^2$	p value
		Frequent	Not frequent		
Symptoms					
Stomach	Yes	1	0	0.286	0.592
ache	No	2	8		
Headache	Yes	2	2	0.331	0.565
	No	1	6		
Lethargy	Yes	0	1	0.0001	1.000
	No	3	7		

$_b$ Chi-Square Test **N=11**

CHAPTER 5

DISCUSSION, CONCLUSON AND RECCOMENDATION

5.1 Discussion

In this study, 215 students were participated to answer the questionnaire in order to collect all the information needed such as respondents' socio demographic data, economic data, health status, other sources that can contribute in getting cadmium into the body, and food frequency questionnaire.

5.1.1 Socio-Demographic Characteristics of Respondents

From the result gathered showed that 20% of male respondents and 71% of female were involved. According to Department of Statistic Malaysia (2010) from Ministry of High Education, numbers of male that enters to high institution was only 35.2% compare to female 72.8%. This shown that it was agreed that number of male was less that female in high institution. There were 14% of the respondents was from

STPM, 75% of the respondents was from Matriculation or Pre-university, and 11% was from Diploma. In this study, majority of the respondents was Malay ethnicity. Department of Statistic Malaysia (2010) surveys shown more than 50% of Malaysia population were Malay ethnicity.

Besides that, majority of the respondents financial sources were from JPA (51%), and followed by PTPTN with 39% and the rest was from parents and other financial sources which totalled 11%. The other financial sources were such as Yayasan Tunku Abdul Rahman and others. The survey done by Department of Statistic of Malaysia (2010) showed majority of Malaysia population age range between 15 to 64 years old. In this study, majority age distribution among the respondents was still in that particular range of age group.

According to Lim (2000) majority of the adult population (61%) had BMI in the normal range, 26.5% had overweight BMI, and 13% had grade thinness. From the result in this study, it was agreed that majority of the respondents had normal BMI. We can assumed that because of the respondents were science students, they will have high awareness on health even though, there were respectively 8% and 4% of respondents who had BMI at overweight and obesity level. This might be because of genetic that they inherit from parents, have bad eating habit or passive contribution in sports.

5.1.2 Respondents Health Information

There were many sign and symptoms that caused by cadmium poisoning such as diarrhea, vomiting, hypertension, kidney damage and many more (Orejimi et al., 2000; Ashraf et al., 2006). The result showed, 3 respondents were having high blood pressure. Other than that, 12% of the respondents had stomach ache, 13% had headache, 8% had lethargy, and 2% had diarrhea and 1% had difficulties to breath. I can assume that, all these respondents that had disease and symptoms were caused by cadmium poisoning. For those 3 respondents that having high blood pressure, their BMI was between normal and underweight range and none of them were smokers. They probably inherit high blood pressure genetic from their parents. In the other hand, high blood pressure was one of the sign showing that they might have chronic kidney disease that can caused by cadmium poisoning in our body.

5.1.3 The Information on Canned Fish Consumed by Respondents

The most favorable types of canned fish consume by respondents were sardine and tuna spread canned fish with respectively 72% consume sardine and 5% consume tuna spread. Tuna spread was the highest being consumed compared to tuna mayonnaise, tuna chili or tuna in water. Sardine and tuna spread had the highest consumption might be because of the taste of this canned fish was tastier compare to the other types of canned fish. It was also easy to use and cheaper to buy. This was agreed because majority of the respondents bought this canned fish from Giant

Supermarket at The Mines and 17 College shop. While for the types of canned fish brand, majority of the respondents had chosen to buy Ayam Brand and King Cup. There were two probably reasons for this selected choice. It might be because of Ayam Brand was known as the most delicious canned fish in the local supermarket in Malaysia. It was also known being in the market for a long time, while for King Cup it's been chosen might be because of the price was much more cheaper compare to Ayam Brand. Nevertheless, King Cup still had a good taste compared to the others canned fish brand in the local market.

5.1.4 The other types of food consumed by respondents

There were 27% of respondents that consume sardine frequently. While for tuna spread there were 3% of respondents whom frequently consume this canned fish. . In Canary Island, fruits, vegetables and cereals were the main food groups contributing to Cd intake (Rubio, 2006). Cadmium was also said present at low levels in most foods, with commodities such as a cereals, fruits, vegetables, meat and fish. This makes it the largest contribution to dietary exposure given the fact that they were also the foodstuffs consumed in largest amounts. Certain wild mushrooms may also contain high levels, as can rice grown in certain geological areas where the soil was rich in cadmium. These foodstuffs were however minor contributors to overall intake of cadmium, as they were eaten relatively in small amounts (Food Safety Authority of Ireland, 2009).

This study found that rice had the highest frequency intake where 94% of the respondents consume it daily, followed by leafy vegetables at 54% of the respondents consume it daily, cereals (17%), shrimp (13%), dark soya sauce (12%) and marine fish (11%). While for the weekly frequency intake showed that 57% of the respondents consume marine fish followed by 41% consumed vegetables, 39% for root vegetables, 36% for anchovies and 32% with leafy vegetables.

5.1.5 Other Possible Sources of Cadmium Exposure to Human

The result showed that there were 6 respondents who were smoking. From the previous study, smoking was known as one of the possible sources of cadmium exposure to human (Rubio, 2005). Furthermore, Johannes (2006) said that, cigarette smoke was the major source of inhalative cadmium intoxication in our body. Human lung can resorbes 40–60% of the cadmium from the tobacco smoke. Besides that, sources of water can also be the possible sources of cadmium. Kavcar et al. (2009) mentioned that cadmium might contain in tap water due to the corrosion of household plumbing system. This study found that, majority of respondent gets their drinking water from tap water (81%).

5.1.6 Concentration of Cadmium in 3 Types of Canned Fish

Cadmium mean concentrations were compared to the Malaysian Food Act, 1983 (Malaysian Food and Drug Regulation 1985). The guideline was produced as guideline for metals concentrations in fish and fish based food. According to the Malaysian Food and Drug Regulation, 1985, maximum allowable concentration of Cd in food and fish product foods was 1.00 mg/kg (Ahmad, 2010). As far as this study was concerned, none of studied canned fish samples exceeds the maximum allowable concentrations. Cadmium was an unessential metal and it presence in ecosystem creates risk of exposure. Although Cd detected in low concentrations, it normally contained in the most mobile fraction in sediment (either exchangeable or carbonate bound) and therefore can easily enter or transported to the food chains (Jain, 2004). Cadmium contamination in fish muscle for Ayam Brand and fish water for King Cup canned fish can caused by pollution of the raw materials and the environment itself or by secondary contamination which caused by poor handling practices of the raw materials, containers and during processing steps neither on land or at sea (Celik, 2005; Tuzen, 2006; Ikem and Egeibor, 2005; Suhendan, 2010). From the previous research, Cd concentration in canned fish ranged from 0.07 to 0.14 microgram per gram (Waqar, 2006). This study reveals that, cadmium concentration in this study is lower than in the previous studies.

5.1.7 Health Risk Assessment of Respondents

In this study, the result obtained showed that HQ for all respondents were less than 1 which indicate it was acceptable as this means that the ADD was below the RfD. While for the LCR result showed that all respondents were having clearly acceptable risk which was less than 1×10^{-6}. This means that an additional one case of cancer was accepted for populations of 1 million or 100,000, respectively. A risk level of 1 in a million, or 1 in one hundred thousand, also implies a likelihood that up to one person out one million or 100,000 equally exposed people would contract cancer if exposed continuously for 24 hours per day to a specific radiation dose over 70 years (an assumed average lifetime) (IRIS, 2009). In this study, the result for ADD and LADD did not exceed the limit for PTWI (0.007mg/kg/bw).

5.1.8 Relationship between the Frequency Intake of Canned Fish and Health Risk Encountered by Respondents

This test was used because the data was between continuous data and interval data that had more than four interval data. The result showed that, there were significant relationships between frequency intake of sardine canned fish and tuna spread canned fish with the health risk encountered by respondents. Both p values were less than 0.0001. Other than that, result for r value showed positive values which indicate there will be a health risk with the increasing of the frequency intake of the canned fish. The higher frequency intake of canned fishes by respondents the higher possible health risk encountered by respondents. Cadmium had a very long half-life which almost 30-40 years. This is the reason why cadmium that entered into our body will stay and accumulate in the body for a long period of time. Other than that, health risk encountered from cadmium contamination was depending on the composition of the diet and the nutritional status of an individual. Flanagan et al. (1998) mentioned that the most important metabolic parameter for cadmium uptake is a person's possible lack of iron. People with low iron supplies showed a 6% higher uptake of cadmium than those with a balanced iron stock (Maduabuchi, 2006). Furthermore a high fibre diet increases the dietary cadmium intake (Jarup, 1998)

5.1.9 Association between Frequency Intake of Canned Fish and Respondents Health Status

While, the results from Chi-Square Test between frequency intake of canned fishes and respondents health statuses showed there were significant between frequency intake of canned fish Sardine and symptom of diarrhea. Nevertheless, we cannot assume that those individuals that had symptom of diarrhea were caused by cadmium poisoning. To ensure it, biological samples such as blood or hair should be taken to get better and more precision result.

5.2 Conclusion and Recommendation

As conclusion, the socio-demographic of the respondents was determined by using pre-tested questionnaire. From the questionnaire, Ayam Brand, King Cup and Ayam Brand Tuna Spread were chosen as the three most favorable canned fish consumed by respondents. In determined the concentration level of cadmium in these canned fish, Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) was used. The concentration level fish muscle for Ayam Brand was the highest and for fish water King Cup had the highest cadmium concentration level mean while tune spread had the lowest cadmium concentration level among all samples. The average of cadmium concentration level were compared to Malaysia Food Act 1983 (Malaysian Food and Drug Regulation 1985) did not exceeded the limt 1 mg/kg.

Average Daily Dose (ADD) and Lifetime Average Daily Dose (LADD) were compared to Provisional Tolerable Weekly Intake (PTWI). None from the ADD and LADD exceed the limit value for PTWI (0.007 mg/kg/bw) established by FAO/WHO. While for Lifetime Excess Cancer Risk (LCR) showed it is clearly acceptable where the value did not exceed 1×10^{-6} and HQ value was in acceptable categories where by the value was less than 1.

The study found, there is significant relationship between frequency intake of canned fish with the health risk of respondents. The results were determined by using nonparametric Spearman's rho test. Mean while for the association between frequency intake of canned fish with respondents health status, there is significant. This study reveals that there is significant association between frequency intake of canned fish sardine with symptoms of diarrhea.

For the overall results, cadmium was present in all canned fish samples but values obtained did not exceed the maximum limit for cadmium by Malaysian Food Act (1 mg/kg). The health risk assessment did not exceed PTWI limits establish by FAO/WHO (2004). Therefore these canned fish are safe to be consumed in terms of these selected Cd concentration. Nevertheless, health risk might encounter with high canned fish consumption. The health risk can be reduced with reduction intake of canned fish and high iron intake in food can help reduce the Cd absorption in our body. Other than that, biological samples such as blood and hair were suggested to be sample to get an accurate and precise result. Further assessment is needed to control the risk of heavy metals in dietary intake. More study on the health risk assessment on canned food consumption is encouraged to provide the date and guideline for human health in Malaysia.

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APPENDIX 1

Medical Research Ethic Committee Approval Letter

APPENDIX 2

Respondent Information Sheets

FAKULTI PERUBATAN DAN SAINS KESIHATAN
FACULTY OF MEDICINE AND HEALTH SCIENCES
UNIVERSITI PUTRA MALAYSIA, 43400 UPM SERDANG, SELANGOR, MALAYSIA

RESPONDENT'S INFORMATION SHEET

Please read the following information carefully, do not hesitate to discuss any questions you may have with your researcher.

STUDY TITLE

DETERMINATION OF CADMIUM CONTAMINATION IN CANNED FISH AND HEALTH RISK ASSESSMENT AMONG STUDENTS OF FACULTY OF MEDICINE AND HEALTH SCIENCES, UNIVERSITI PUTRA MALAYSIA

INTRODUCTION

This study is to determine level of cadmium in local canned fish and health risk assessment among students in Faculty of Medicine and Health Sciences, Universiti Putra Malaysia will be done.

WHAT WILL YOU HAVE TO DO?

Respondents need to answer the questionnaire that contain background and socio-demografic information, exposure on cadmium, health information, and the food frequency information. After respondents finish answering the questionnaire, respondents need to handback the questionnaire paper and before going out from the room respondents height and weight will be taken.

WHO SHOULD NOT ENTER THE STUDY?

-Individuals age below 19 years old and individuals above 30 years old.

- Individuals that do not staying in College 17, UPM.

- Individuals that do not eat canned fish in their diet intake.

WHAT WILL BE BENEFITS OF THE STUDY:

(a) TO YOU AS THE SUBJECT?

This study is providing the information on canned fish in local market that you can easily get and the one that you might always consume. This canned food might contain heavy metals that can effect to our health. So, in this study, as the respondents will know more information on this type of food and its benefits and adverse effect of consuming.

b) TO THE INVESTIGATOR?

This study will be providing the new data base in Malaysia on heavy metals in canned fish and the health risk assessment for future review.

ARE THERE ANY RISKS?

The will be no risk in this study to the respondents.

WILL THE INFORMATION AND MY IDENTITY REMAIN CONFIDENTIAL?

All details information of respondents in this study will be private and confidential and will be used only in this study not for public review.

WHO SHOULD I CONTACT IF I HAVE ADDITIONAL QUESTIONS DURING THE COURSE OF THE RESEARCH?

LISA PUTRIKU DIANA BINTI AB KAHAR
RESEARCHER
BAC. SC. ENVIRONMENTAL AND OCCUPATIONAL HEALTH

APPENDIX 3

Respondent Informed Consent Form

APPENDIX 4

Questionnaire

No. Responden : | | | |

TAJUK KAJIAN:

PENENTUAN PENCEMARAN KADMIUM PADA IKAN DALAM TIN DAN PENILAIAN RISIKO KESIHATAN DI KALANGAN PELAJAR FAKULTI PERUBATAN DAN SAINS KESIHATAN, UNIVERSITI PUTRA MALAYSIA

BORANG SOAL SELIDIK

Nama Penemuduga :	
Tarikh :	

Semua maklumat yang diperolehi dari borang soal selidik ini adalah **SULIT** dan adalah untuk kegunaan di dalam kajian ini sahaja. Borang soal selidik ini mesti dilengkapkan sepenuhnya. Terima kasih di atas kerjasama anda.

Respondent ID:

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UNIVERSITI PUTRA MALAYSIA, 43400 UPM SERDANG, SELANGOR, MALAYSIA

BAHAGIAN 1	KETERANGAN DIRI	RUANGAN KOD

Tandakan (√) dalam kotak berkenaan.
* Potong pada yang tidak berkenaan.

Nama Penuh: ....

* Kursus: BS KPP / BS PKK / BS BIOMEDICAL / BS DIETETIK

1. Umur: .... tahun
2. Jantina: ☐ Lelaki ☐ Perempuan
3. Berat: ___________ kg
4. Tinggi: ___________ cm
5. Tekanan darah: ___________________
6. Bangsa: ☐ Melayu ☐ Cina ☐ India
   ☐ Lain-lain (Sila nyatakan): ....
7. Agama:
   ☐ Islam
   ☐ Kristian
   ☐ Buddha
   ☐ Hindu
   ☐ Lain-lain
8. Taraf pendidikan:
   ☐ STPM
   ☐ Matrikulasi
   ☐ Diploma
   ☐ Lain-Lain: (Sila nyatakan): ....
9. Sumber kewangan:
   ☐ JPA
   ☐ PTPTN
   ☐ Ibubapa
   ☐ Lain-lain: (Sila nyatakan): ....

Respondent ID:

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UNIVERSITI PUTRA MALAYSIA, 43400 UPM SERDANG,
SELANGOR, MALAYSIA

10. Perbelanjaan bulanan keseluruhan (RM) : _______________

11. Perbelanjaan bulanan bagi makanan sahaja (RM) : ______________

12. Tempoh telah menetap di Kolej 17: _________ bulan / tahun

BAHAGIAN 2	MAKLUMAT KESIHATAN	RUANGAN KOD

11. Adakah anda mengalami masalah kesihatan berikut?

- [ ] Kanser, nyatakan : ___________________
- [ ] Diabetes/kencing manis
- [ ] Darah tinggi
- [ ] Penyakit buah pinggang
- [ ] Penyakit jantung
- [ ] Pekak
- [ ] Bisu
- [ ] Terlantar sakit teruk
- [ ] Penyakit lain, nyatakan : ___________________

12. Adakah anda sering mengalami simptom-simptom seperti berikut?

- [ ] Sakit perut
- [ ] Muntah
- [ ] Cirit-birit
- [ ] Kekejangan otot
- [ ] Pening
- [ ] Kesukaran bernafas
- [ ] Kelesuan

Respondent ID:

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BAHAGIAN 3:	ANTHROPOMETRI	RUANGAN KOD
1. Nama peserta :	1.	
2. Berat (kg) :	2. i. ☐☐☐ . ☐ kg ii. ☐☐☐ . ☐ kg iii. ☐☐☐ . ☐ kg	
3. Tinggi (cm) :	3. i. ☐☐☐ . ☐ kg ii. ☐☐☐ . ☐ kg iii. ☐☐☐ . ☐ kg	

Pengukuran Indeks Jisim Tubuh (IJT) / *Body Mass Index (BMI)*

BMI = Berat (kg) / Tiggi x Tinggi(m2)

Klasifikasi ⇨

IJT / BMI < 18.5 = Kurang Berat Badan
IJT / BMI 18.5 - 24.9 = Normal
IJT / BMI 25.0 - 29.9 = Berlebihan berat badan
IJT / BMI ≥ 30.0 = Obes

Respondent ID:

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SELANGOR, MALAYSIA

BAHAGIAN 4:	PENDEDAHAN CADMIUM DALAM MAKANAN	RUANGAN KOD

Tandakan (√) dalam kotak berkenaan.

1. Dimanakah anda selalu mendapatkan makanan harian anda?

   i. Membeli di kafeteria Kolej 17 ☐
   ii. Membeli di luar Kolej 17 ☐
   iii. Memasak sendiri ☐
   iv. Lain-lain ☐
   (Sila nyatakan):____________________

2. Adakah anda makan ikan dalam tin?

   i. Ya ☐ ii. Tidak ☐

3. Apakan jenis ikan dalam tin yang anda makan?

   i. Ikan Sardin ☐
   ii. Ikan Keli ☐
   iii. Ikan Mackerel ☐
   iv. Ikan Tuna ☐
   v. lain-lain ☐
   (Sila nyatakan): ____________________

4. Dimanakah anda selalu makan ikan dalam tin?

   i. Kafeteria Kolej 17 ☐
   ii. Membeli di luar Kolej 17 ☐
   iii. Memasak sendiri ☐
   iv. Lain-lain ☐
   (Sila nyatakan): ____________________

Respondent ID:

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5. Apakah jenama ikan dalan tin yang anda beli?

i. Ayam Brand ☐
ii. Adabi ☐
iii. Marina ☐
iv. Lain-lain ☐
(Sila nyatakan): ____________________

6. Dimanakah anda selalu membeli ikan dalam tin?

i. Koperasi Kolej 17 ☐
ii. Pasaran Tempatan ☐
Nyatakan: ____________________
iii. Kedai runcit Sri Serdang ☐
iv. Lain-lain ☐
(Sila nyatakan): ____________________

Respondent ID:

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BAHAGIAN 5: BORANG KEKERAPAN PENGAMBILAN MAKANAN

Kod	Jenis makanan	Kekerapan pengambilan					Ukuran sajian(pilih satu jenis ukuran sahaja)	Berapa banyak sajian setiap kali makan
	(A)Bijiran dan hasil bijiran	Berapa kali sehari	Berapa kali seminggu	Berapa kali sebulan	Berapa kali setahun	Tidak makan		
A1	Nasi						Pinggan	
							Mangkuk cina	
							Cawan	
							senduk	
A2	Mee kuning/mee segera						Pinggan	
							Mangkuk cina	
							Cawan	
							senduk	
A3	Mihun/kuew teow/laksa /laksam						Pinggan	
							Mangkuk cina	
							Cawan	
							senduk	
A4	Roti						Pinggan	
							Mangkuk cina	
							Cawan	
							senduk	
A5	Bijirin sarapan pagi						Pinggan	
							Mangkuk cina	
							Cawan	
							senduk	

Respondent ID:

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Kod	Jenis makanan	Kekerapan pengambilan					Ukuran sajian(pilih satu jenis ukuran sahaja)	Berapa banyak sajian setiap kali makan
	(B)Ikan dan makanan laut	Berapa kali sehari	Berapa kali seminggu	Berapa kali sebulan	Berapa kali setahun	Tidak makan		
B1	Ikan laut						Keping	
							Ekor	
B2	Ikan air tawar						Keping	
							Ekor	
B3	Ikan bilis						Sudu makan	
B4	Kekerang						Sudu makan	
B5	Udang basah						Ekor sederhana	
B6	Sotong basah						Potong sederhana	
B7	Sotong kering						Keping sederhana	
							Potong sederhana	
B8	Ketam						Ekor	
B9	Ikan kering						Keping	
							Ekor	
B10	Bebola ikan/kek ikan						Bebola	
							Ketul	
B11	Keropok lekor						Ketul	

Kod	Jenis makanan	Kekerapan pengambilan					Ukuran sajian(pilih satu jenis ukuran sahaja)	Berapa banyak sajian setiap kali makan
	(C)Kekacang dan hasilnya	Berapa kali sehari	Berapa kali seminggu	Berapa kali sebulan	Berapa kali setahun	Tidak makan		
C1	Kekacang						Sudu makan	
C2	Tauhu						Keping	
C3	Tempe						Keping	
							Sudu makan	
C4	Kacang Tanah						Sudu makan	

Respondent ID:

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Kod	Jenis makanan	Kekerapan pengambilan					Ukuran sajian (pilih satu jenis ukuran sahaja)	Berapa banyak sajian setiap kali makan
	(D)Sayuran	Berapa kali sehari	Berapa kali seminggu	Berapa kali sebulan	Berapa kali setahun	Tidak makan		
D1	Sayuran berdaun						Cawan	
D2	Sayuran kacang						Cawan	
D3	Sayuran berubi						Cawan	
D4	Sayuran kobis						Cawan	
D5	Petola/labu /timun						Cawan	
D6	Ulam-ulaman						Cawan	
D7	Putik jagung						Sudu makan	
D8	Cendawan basah /kering						Cawan	
D9	Taugeh						Cawan	

Kod	Jenis makanan	Kekerapan pengambilan					Ukuran sajian(pilih satu jenis ukuran sahaja)	Berapa banyak sajian setiap kali makan
	(E)Makanan perencah /perasa	Berapa kali sehari	Berapa kali seminggu	Berapa kali sebulan	Berapa kali setahun	Tidak makan		
E1	Gula						Sudu teh	
E2	Madu						Sudu teh	
E3	Belacan						Sudu makan	
E4	Cencalok						Sudu teh	
E5	Budu						Sudu teh	
E6	Kicap pekat						Sudu teh	
E7	Kicap cair						Sudu makan	
E8	Sos cili/tomato						Sudu makan	
E9	Sos tiram						Sudu teh	
E10	Sos ikan						Sudu teh	
E11	Otak udang						Sudu teh	

Respondent ID:

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Kod	Jenis makanan	Kekerapan pengambilan					Ukuran	Berapa
	(F)Makanan yang dikaji **Senaraikan jenis ikan dalam tin yang paling kerap anda makan**	**Berapa kali sehari**	**Berapa kali seminggu**	**Berapa kali sebulan**	**Berapa kali setahun**	**Tidak makan**	**sajian(pilih satu jenis ukuran sahaja)**	**banyak sajian setiap kali makan**
F1							ekor	
F2							ekor	
F3							ekor	
F4							ekor	
F5							ekor	

Sudah berapa lamakah anda mengambil makanan ikan dalam tin sebagai salah satu menu harian?

Sila nyatakan : ________________________________ hari/ bulan/ tahun

Respondent ID:

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BAHAGIAN 6:	FAKTOR-FAKTOR PENDEDAHAN LAIN	RUANGAN KOD
Tandakan (√) dalam kotak berkenaan.		

Sumber bekalan air

1. Dari manakah anda mendapat sumber bekalan air minuman?

   ☐ Air paip
   ☐ Air perigi
   ☐ Lain-lain :_______________

Amalan gaya hidup

a) Merokok

2. Adakah anda merokok?

   ☐ Ya
   ☐ Tidak
   ☐ Sudah berhenti

   Jika Ya, sila nyatakan berapa tahun anda sudah merokok dan bilangan batang rokok dihisap dalam sehari:

   Bil.tahun: ___________ Bil. Batang rokok sehari _________

b) Pengambilan alkohol

3. Adakah anda pernah mengambil minuman beralkohol?

   ☐ Ya
   ☐ Tidak

   Jika ya, sila nyatakan berapa botol sehari anda minum?

   __________________ botol

APPENDIX 5

Gantt Chart

GANNT CHART: RESEARCH SHEDULE

Activity	2011				2012					
	9	10	11	12	1	2	3	4	5	6
Study Approval										
Screening (Questionnaire)										
Data Collection										
Analysis Data										
Report Writing										

APPENDIX 6

Results of Samples Analysis

Daily Performance Report

Sample ID: Smart Tune Solution
Sample Date/Time: Wednesday, March 28, 2012 11:32:31
Sample Description: Performance check
Method File: C:\Elandata_LC-ICPMS\Method\daily performance.mth
Dataset File: C:\Elandata_LC-ICPMS\Dataset\daily performance\Smart Tune Solution.256
Tuning File: C:\Elandata_LC-ICPMS\Tuning\default.tun
Optimization File: C:\Elandata_LC-ICPMS\Optimize\default.dac
Dual Detector Mode: Pulse

Acq. Dead Time(ns): 55
Current Dead Time (ns): 55

Summary

	Analyte	Mass	Meas. Intens. Mean	Net Intens. Mean	Net Intens. SD	Net Intens. [illegible]SD
	Mg	24.0	77734.2	77734.190	1189.049	1.5
	In	114.9	272861.7	272861.704	5849.314	2.1
	U	238.1	349530.7	349530.687	7137.07[illegible]	2.0
[>	Ce	139.9	244612.1	244612.138	5946.389	2.4
[	CeO	155.9	4022.0	0.016	0.001	4.6
[>	Ba	137.9	196406.8	196406.801	36[illegible]90	1.8
[	Ba++	69.0	4820.6	0.025	0.00[illegible]	2.7
	Bkgd	220.0	7.9	7.900	[illegible]03	8.8
	Bkgd	8.5	17.4	17.433	2.01[illegible]	11.6

Current Optimization File Data

Current Value	Description
0.72	Nebulizer Gas Flow [NEB]
1.20	Auxiliary Gas Flow
17.00	Plasma Gas Flow
8.00	Lens Voltage
1100.00	ICP RF Power
-1700.00	Analog Stage Voltage
750.00	Pulse Stage Voltage
0.00	Quadrupole Rod Offset Std [[illegible]RO]
-12.00	Cell Rod Offset Std [CRO]
25.00	Discriminator Threshold
-26.00	Cell Path Voltage Std [C[illegible]
0.00	RPa
0.25	RPq
0.91	DRC Mode NEB
-5.50	DRC Mode QRO
-0.50	DRC Mode CRO
-16.00	DRC Mode C[illegible]
0.00	Cell Gas A

Current Autolens D[illegible]a

Analyte	Mass	Num o[illegible]	D[illegible] Value	Maximum Intensity
Be	9	45	6.5	4441.1
Co	59	45	7.3	115569.0
In	115	45	8.3	241458.6

Quantitative Analysis - Summary Report

Sample ID: 19 (sampel 2) (kiry cup)

Sample Date/Time: Wednesday, March 28, 2012 12:49:42
Sample Description: Food samples
Solution Type: Sample
Blank File:
Number of Replicates: 3
Peak Processing Mode: Average
Signal Profile Processing Mode: Average
Dual Detector Mode: Dual
Dead Time (ns): 55

Sample File: C:\Elandata_LC-ICPMS\Sample\Nor Aisyah (FPSK).sam
Method File: C:\Elandata_LC-ICPMS\Method\nor aisyah (fpsk).mth
Dataset File: C:\Elandata_LC-ICPMS\DataSet\Nor Aisyah (FPSK)\19.029
Tuning File: C:\Elandata_LC-ICPMS\Tuning\default.tun
Optimization File: C:\Elandata_LC-ICPMS\Optimize\default.dac
Calibration File: C:\Elandata_LC-ICPMS\System\Nor Aisyah (FPSK).cal
Calibration Type: External Calibration

Summary

Intensities

Analyte	Mass	Meas. Intens. Mean	Meas. Intens. RSD	Blank Intensity	Blank Intens. RSD
As	75	113308	[illegible]590	[illegible].003	7.911
Cd	111	1241	[illegible].469	113.334	14.264
Pb	208	[illegible]4923	3.409	200.002	10.149

Concentration Results

Analyte	Mass	Net Intens. Mean	Conc. Mean	Conc. SD	Conc. RSD	Sample Unit
As	75	[illegible]13091.[illegible]8	**86.601**	1.38	1.6	ppb
Cd	111	1128.084	**0.648**	0.03	4.9	ppb
Pb	208	24723.476	**1.469**	0.05	3.4	ppb

J: 0.015552 mg/kg.

Quantitative Analysis - Summary Report

Sample ID: 20 (sample 7) (king cup)

Sample Date/Time: Wednesday, March 28, 2012 12:51:26
Sample Description: Food samples
Solution Type: Sample
Blank File:
Number of Replicates: 3
Peak Processing Mode: Average
Signal Profile Processing Mode: Average
Dual Detector Mode: Dual
Dead Time (ns): 55

Sample File: C:\Elandata_LC-ICPMS\Sample\Nor Aisyah (FPSK).sam
Method File: C:\Elandata_LC-ICPMS\Method\nor aisyah (fpsk).mth
Dataset File: C:\Elandata_LC-ICPMS\DataSet\Nor Aisyah (FPSK)\20.030
Tuning File: C:\Elandata_LC-ICPMS\Tuning\default.tun
Optimization File: C:\Elandata_LC-ICPMS\Optimize\default.dac
Calibration File: C:\Elandata_LC-ICPMS\System\Nor Aisyah (FPSK).cal
Calibration Type: External Calibration

Summary

Intensities

Analyte	Mass	Meas. Intens. Mean	Meas. Intens. RSD	Blank Intensity	Blank Intens. RSD
As	75	112363	2.484	216.003	7.911
Cd	111	1309	4.077	113.334	14.264
Pb	208	23734	[illegible].983	20[illegible].002	10.149

Concentration Results

Analyte	Mass	Net Intens. Mean	Conc. Mean	Conc. SD	Conc. RSD	Sample Unit
As	75	112147.080	[illegible]85.877	2.14	2.5	ppb
Cd	111	1196.094	[illegible].687	0.03	4.5	ppb
Pb	208	2[illegible].291	1.398	0.04	3.0	ppb

= 0.016488 mg/kg