



**UNIVERSITI PUTRA MALAYSIA**

***ARSENIC CONCENTRATION IN SHRIMP PASTE AND HEALTH RISK  
ASSESSMENT IN TWO VILLAGES OF MELAKA***

**BY  
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## ABSTRACT

ARSENIC CONCENTRATION IN SHRIMP PASTE AND HEALTH RISK  
ASSESSMENT IN TWO VILLAGES IN MELAKA.

AZIEMAH BINTI ZULKIFLI

**Introduction:** Arsenic (As) is a naturally occurring element widely distributed in the earth's crust and human are affected by exposure to arsenic in food chain and drinking water. **Objective:** A cross-sectional study was conducted at Kampung Pantai Rombang (PR) and Kampung Pinang (KP), Melaka to determine the arsenic concentration in shrimp paste and perform health risk assessment estimated among respondent. This study also aimed to determine the relationship between shrimp paste frequency intake with health risk which indicated by Hazard Quotient (HQ) and Lifetime Cancer Risk (LCR) and to determine relationship between shrimp paste frequency intake and health symptoms of respondents. **Material & Methodology:** A total of 151 respondents were randomly selected based on inclusion criteria. 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 the Average Daily Dose (ADD), Lifetime Average Daily Dose (LADD), frequency of shrimp paste intake and health symptoms experienced by respondents. Two duplicates of shrimp paste samples were purchased from each village. The arsenic concentration was analyzed by using Inductive Couple Plasma Mass Spectrophotometer (ICP-MS). **Results:** All shrimp paste samples contained As which exceeded the maximum As concentration level stated in Malaysia Food Regulation 1985(1 mg/kg). The finding on LCR and HQ demonstrated that all respondents encountered acceptable risk towards carcinogenic and noncarcinogenic health risk. On the other hand, the result also depicted that there were no significant relationship between the shrimp paste frequency intake with LCR and HQ. The result also exhibits no significant association between shrimp paste frequency intake with acute and chronic As poisoning signs. **Conclusion:** As was detected in all shrimp paste samples at concentration which exceeded the permitted As concentration in food. There was unlikely potential adverse health effects from arsenic intake in shrimp paste in this study. It is recommended that the respondents undergo biological monitoring in order to determine the actual As exposure and associate it with health risk encountered by the study population.

**Key words:** Arsenic, Shrimp paste, Health Risk Assessment, LADD, ADD, HQ, LCR

## ABSTRAK

### KEPEKATAN ARSENIK DI DALAM BELACAN DAN KAJIAN RISIKO KESIHATAN DI DUA KAMPUNG DI MELAKA

AZIEMAH BINTI ZULKIFLI

**Pengenalan:** Arsenik(As) adalah element semulajadi yang tersebar dengan luas di kerak bumi dan manusia boleh terdedah kepada As melalui rantai makanan dan air minuman. **Objektif:** Kajian keratan rentas ini telah dijalankan di Kampung Pantai Rombang dan Kampung Pinang, Melaka yang bertujuan untuk menentukan kepekatan As yang terkandung di dalam sampel belacan, menjalankan penilaian risiko kesihatan di kalangan responden, menentukan kaitan antara kekerapan pengambilan belacan dengan risiko kesihatan yang ditentukan oleh *HQ* dan *LCR* dan untuk menentukan kaitan antara kadar pengambilan belacan dengan tanda-tanda masalah kesihatan yang berkaitan dengan pendedahan terhadap As. **Bahan dan Metodologi:** Seramai 151 orang responden telah dipilih secara rawak berdasarkan kriteria tertentu. Satu set soal selidik digunakan untuk mendapatkan informasi social-demografi dan menilai risiko kesihatan yang dihadapi oleh responden menggunakan *ADD*, *LADD*, kekerapan pengambilan belacan, dan tanda-tanda masalah kesihatan yang dialami oleh responden. Sebanyak 5 sampel belacan diperolehi dari kedua-dua kampung. Kepekatan As di dalam belacan telah di analisis dengan menggunakan *ICP-MS*. **Hasil kajian:** Semua sampel belacan mengandungi kandungan As yang melebihi tahap maksimum As di dalam makanan yang dibenarkan dalam Peraturan-Peraturan Makanan 1985 iaitu 1 mg/kg. Keputusan mendapati *HQ* dan *LCR* semua responden berada pada tahap yang boleh diterima terhadap risiko kesihatan kanser dan bukan kanser. Hasil kajian menunjukkan bahawa tiada hubungan antara kekerapan pengambilan belacan dengan risiko kesihatan. Keputusan kajian juga menunjukkan bahawa tiada perkaitan antara kekerapan pengambilan belacan dan tanda-tanda masalah kesihatan berpunca daripada keracunan As. **Kesimpulan:** As dikesan dalam setiap sampel belacan pada kepekatan melebihi paras yang dibenarkan. Pengambilan belacan tidak berpotensi untuk mendatangkan kesan kesihatan yang teruk kepada penduduk. Responden dicadangkan supaya menjalani pemantauan biologi untuk menentukan tahap pendedahan yang sebenar kepada As and mengaitkannya dengan risiko kesihatan.

**Kata kunci:** Arsenik, belacan, penilaian risiko kesihatan, *ADD*, *LADD*, *HQ*, *LCR*

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## LIST OF ACRONYMS AND ABBREVIATIONS

As	Arsenic
ADD	Average Daily Dose
ATSDR	Agency for Toxic Substances and Disease Registry
CCA	Copper chromate arsenate
$\mu\text{g/L}$	Microgram per litre
GFAAS	Graphite furnace atomic absorption spectrometry
HQ	Hazard Quotient
ICP-MS	Inductively coupled plasma mass spectrometry
JECFA	Joint FAO/WHO Expert Committee on Food Additives
KP	Kg. Pinang
LADD	Lifetime Average Daily Dose
LCR	Lifetime Cancer Risk
$\text{mg/kg}$	Milligram per kilogram
ppb	Part per billion
PR	Kg. Pantai Rombang
PTWI	Provisional Tolerable Weekly Intake



## CHAPTER 1

### INTRODUCTION

#### 1.1 Background

##### 1.1.1 Arsenic

Arsenic is a naturally occurring element widely distributed in the earth's crust. In the environment, arsenic is combined with oxygen, chlorine, and sulphur to form inorganic arsenic compounds. Arsenic in animals and plants combines with carbon and hydrogen to form organic arsenic compounds. Inorganic arsenic compounds are mainly used to preserve wood. Copper chromate arsenate (CCA) is used to make "pressure-treated" lumber. Organic arsenic compounds are used as pesticides, primarily on cotton fields and orchards (ATSDR, 2007).

##### 1.1.2 Arsenic Sources

Arsenic is part of the natural environment and is contained in soil and rock (ATSDR, 2007). Arsenic is found in the natural environment in some abundance in the earth's

crust and in small quantities in rock, soil, water and air. It is present in many different minerals. About one third of the arsenic in the atmosphere comes from natural sources, such as volcanoes, and the rest comes from man-made sources. Due to natural geological contamination, high levels of arsenic can be found in drinking water that has come from deep drilled wells. This is particularly true for Bangladesh (GreenFacts, 2004).

Environmental contamination also occurs because it is used in agricultural pesticides and in chemicals for timber preservation. Arsenic occurs in different forms and some is transported between different parts of the environment where it may change its form. Arsenic in weathered rock or soil can be picked up and moved by the wind and water. Many arsenic compounds bind to soil and only move short distances when water percolates down through the soil. If arsenic is released into the atmosphere by industrial processes or volcanic activity, it attaches to particles that are dispersed by the wind and fall back to the ground. Microbes in soil and sediment also release substances containing arsenic into the atmosphere. These are then converted to other arsenic compounds that settle back onto the ground (IPCS, 2001).

The accumulation of trace elements in environmental samples (soil, sediment, water, biota, etc.) can cause a potential risk to human health due to the transfer of these elements in aquatic media, their uptake by plants and subsequent introduction into the food chain. It is therefore necessary to monitor human exposure to toxic

trace elements present in the food chain, and a number of studies have reported the total arsenic content of foodstuffs from different countries (Dabeka *et al.*, 1993; Tsuda *et al.*, 1995; Sapunar-Postruznik *et al.*, 1996; Roychowdhury *et al.*, 2002, 2003; Meharg and Mazibur, 2003; Alam *et al.*, 2003; Das *et al.*, 2004). According to Rasmussen., (2004) on Environmental Health and Human Exposure Assessment, stated that the inorganic forms of arsenic, which are the dominant forms in surface and groundwater, are the most toxic forms, while the organic forms, common in fish products, are much less toxic. Humans primarily take up arsenic from drinking water and food products.

### 1.1.3 Health Effects

Inhalation of inorganic arsenic may cause respiratory irritation, nausea, skin effects, and increased risk of lung cancer. Acute high dose oral exposure to inorganic arsenic may cause nausea, vomiting, diarrhea, cardiovascular effects and encephalopathy. Long term oral exposure to low levels of inorganic arsenic may cause dermal effects (such as hyperpigmentation and hyperkeratosis, corns and warts) and peripheral neuropathy characterized by a numbness in the hands and feet that may progress to a painful “pins and needles” sensation. There may also be an increased risk of skin cancer, bladder cancer, and lung cancer. Oral exposure to MMA may result in gastrointestinal damage. Kidney effects may be observed following chronic exposure. Chronic oral exposure to DMA may result in urinary bladder and kidney effects. Children who are exposed to high levels of arsenic exhibit symptoms similar to those seen in adults, including cardiovascular, dermal, and neurological effects,

and vomiting following ingestion. There is some evidence that metabolism of inorganic arsenic in children is less efficient than in adults (ATSDR, 2007).

#### 1.1.4 Shrimp paste

Malaysian locally processed raw food products are widely used as main ingredients in local cooking (Sharif *et al.*, 2007). Shrimp paste or 'belacan' as it is called in Malay is a popular food ingredient in Malaysia. It is made from fresh tiny shrimps known as '*geragau*' (Appendix II: Figure 1.1). Traditionally, shrimp paste is used as a food enhancer in countless Malaysian dishes (Leong *et al.*, 2009). Shrimp paste is commonly sold in dried blocks that range in color from pink to dark brown.

Generally, shrimp paste is roasted prior to usage, either wrapped in foil or dry roasted in a wok, toasted over a gas flame on the back of a spoon or by using a fork. This is to enhance the flavour as well as killing bacteria (Hutton, 2005). With the addition of shrimp paste, it will make the food more flavourful and appetizing as shrimp paste is perceived to contain a special taste that can make a dish more palatable and delicious. Thus it has become an essential ingredient in curries and dipping sauces for a more appealing taste (Leong *et al.*, 2009).



Figure 1.2: Shrimp paste

The production begins as soon as the shrimps arrived at the seashore. There are various methods of processing shrimp paste. One of the methods will be explained here. First, the shrimps are mixed with salt and immediately dried under the sun for half a day or one whole day. The semi-dried salted shrimps are pounded; at this point the paste mixture is placed in a tightly sealed container and kept for seven days at room temperature to allow fermentation to take place. The fermented shrimp paste will be dried, mashed and kept for several days until a desired textured is achieved. Finally, the paste will be formed into different shapes and sizes (Adnan, 1984).

The nutritional composition of shrimp paste varies as revealed in some studies. According to Sharif *et al.* (2007), the protein content of shrimp paste was

found to be high, 3 approximately around 28-40%. This value was found to be higher as compared to the protein in meat (25-32%), fish (25-29%), milk (9-10%) and peas (5-8%) (Adnan, 1984). On the other hand, Peralta *et al.* (2005) discovered that the fermented shrimp paste contained anti-oxidative substances. In addition, they also confirmed that there was a significant large amount of polyunsaturated fatty acids (PUFA) and free amino acids. Thus this implies that the antioxidative substances in the fermented shrimp paste can prevent the PUFA from undesirable lipid peroxidation. Peralta *et al.*, (2005) also stressed that the salt-fermented shrimp paste will serve as an effective antioxidant in the human body when included into human's daily diet. Shrimp paste can also be a good source of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA).

However, proximate analysis which was used in the study by Sharif *et al.*, (2007) for the evaluation of macronutrients in food showed that shrimp paste did not comply with the protein requirement (<25%) as in Food Act 1983. Salt was found in every sample with the highest percentage being detected in shrimp paste which exceeded 20%. Following heavy metal analysis (arsenic, cadmium, lead and mercury), arsenic was found in every sample with dried shrimps showing the highest value as compared to the other samples (6.16 mg/kg). The study by Sharif *et al.*, (2007) concluded that several food extracts showed cytotoxic effect but did not cause DNA damage against Chang liver cells.

Besides, salt was found as the main additive and arsenic was present in every sample, which could be the probable cause of the toxicity effects observed (Sharif *et al.*, 2007). In spite of that, diet plays a major role in cancer etiology and prevention (AICR, 1997). Small shrimp called as *geragau* was the main ingredient in shrimp paste production. Hence, there was possibility of arsenic contamination from that marine organism. According to Attar (1992) arsenic levels in marine organism are influenced by species differences, size of organism, and human activities. Bottom dwellers such as shrimp, crab, and lobster accumulated more arsenic 4 than fish due to their frequent contact with bottom sediments. Although the arsenic-contaminated shrimp paste was consumed in small dose, it was taken continuously. Hence, the risk of community to get adverse health effects due to arsenic poisoning might be increased.

## 1.2 Problem Statement

Based on the local previous study on toxicological evaluation of some Malaysian locally processed raw food products, arsenic was found in every sample with dried shrimps showing the highest value as compared to the other samples (6.16 mg/kg) (Sharif *et al.*, 2005). The finding creates a health concern particularly in community health effects since the shrimp paste was the most favourable condiment among Malaysian regardless age and races.

According to several studies, it was stated that the As exposure mostly come from food chain. According to International Programme on Chemical Safety (IPCS) on title "Environmental Health Criteria for Arsenic and Arsenic Compounds" reported that most arsenic in the body comes from the diet. Carbonell-Barrachina *et al.*, (2009) revealed that nearly 100 million rural people in Asia were affected by exposure to arsenic in food chain and drinking water. Besides that, it was found by Borak *et al.*, (2007), seafood was the largest dietary source of As in US. Higher levels of arsenic tend to be found in seafood, rice, grains, and flour (Tara *et al.*, 2008). All these showed that, As exposure to community mostly originated from food sources.

Adverse toxic effects of arsenic as well as its widespread distribution in the environment raise concern about levels of arsenic in man's diet. The contamination of As in shrimp paste might cause by contamination of "geragau" shrimp as shrimp paste main ingredients. It might possible as there were few studies reported of As contamination in marine organisms. Higher levels of arsenic in the diet can result in a higher accumulation rate (Bou-Olayan *et al.*, 1995). Attar *et al.*, (1992) found that As levels in marine organisms were influenced by species differences, size of organism, and human activities. Bottom dwellers such as shrimp, crab, and lobster accumulate more arsenic than fish due to their frequent contact with bottom sediments. Edible seaweeds can also be relatively high in arsenic, accumulating levels of about 200 mg kg dry mass or several orders of magnitude greater than the surrounding water. This

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arsenic is absorbed by the body as demonstrated by the appearance of arsenic compounds in blood and urine following consumption of seaweed (VanHulle *et al.*, 2004). Hence, it proved that the marine organism such as seafood prone to get contaminated with the heavy metal like arsenic.

Based on study by Sharif *et al.*, (2005), they found that As was present in every sample, which could be the probable cause of the toxicity effects observed (Sharif *et al.*, 2005). The toxic nature of arsenic is such that chronic exposure to the element can lead to internal cancers of the bladder and kidney, skin cancer, neurological effects, and cardiovascular disease. Toxic elements, particularly arsenic, which may be present in food, are biologically important at very low concentrations. The U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry (ATSDR) defines a minimal risk level for chronic inorganic arsenic exposure to be 0.0003 mg As/kg/day (ATSDR, 2007). In conclusion, it clearly showed that the extent of community exposure to As in diet should be emphasized in preventing adverse health effects to the community in the future.

### 1.3 Study Justification

Malaysia as the country enriched with the local processed food which contained seafood-based ingredients may be affected or exposed to the heavy metal contaminants in the food products. Based on the previous study which found that

every of dried shrimps showing the highest value of arsenic content as compared to other sample, it depicted that there are possibility of shrimp-based processed food like shrimp paste to be contaminated with the arsenic (Sharif *et al.*, 2005). Hence, the shrimp paste from main production state of this food product shall be analyzed for arsenic contents.

Melaka is the well known state in producing the high quality of shrimp paste in Malaysia. The industry may include the Small Medium Industry (SMI) and big scale industry. Instead of the differences in the size of business, there may also have the differences in food products' process, management, quality control and environment which can contribute to the sources of arsenic contaminant in the shrimp paste. Contact between food and the coat metal surface of packing containers or the processing equipment is a significant source of toxic contamination in food. Thus, the presence of these elements in crops originating, for example, directly from harvest, can undergo a change owing to the effects of food processing (Zukowska and Biziuk, 2008).

Nowadays, the shrimp paste already becomes the common ingredients or dishes to be prepared in the meal of the Malaysian community. According to the study on Toxicological evaluation of some Malaysian locally processed, the level of arsenic content in the shrimp paste exceed the permitted arsenic level in food product stated in the Malaysia Food Act 1983 which is 1 mg/kg. However, there still in

question on how extent is the health risk encounter by the consumer or community who consume the shrimp paste in their daily diet intake.

In order to assess public health impacts associated with arsenic in shrimp paste, it is important to estimate human exposure to the metal via shrimp paste consumption. Hence, the health risk assessments of the community shall be conducted by accessing their frequency of consumption and also the quantity of dietary intake of shrimp paste. Indirectly, health risk from the intake of shrimp paste in meals of community can be estimated.

All these shows that this study is reasonable to be carried out in accordance to assess the arsenic exposure through the consumption of local processed shrimp paste products.

#### **1.4 Conceptual Framework**

Figure 1.3 shows the conceptual framework of arsenic exposure which can be exposed by human. A person can get exposure to arsenic through various ways. The sources can come from the environmental, occupational exposure and from dietary habit. In the environment, arsenic occurs naturally in soil and minerals and may

enter the air, water, and land from wind-blown dust and may get into water from runoff and leaching. Indirectly, the water sources become contaminated with the arsenic and get into the human.

Besides, the human also can be exposed to the arsenic through the occupational field. For example, arsenic is emitted as a fine dust when arsenic-containing ores are heated at smelters to process copper or lead. However, according to the recent study, human are mainly exposed to the arsenic through their dietary intake (ATSDR, 2000). Basically, arsenic mainly gets rid in the seafood when there are contaminations of water sources with arsenic. Since the variety of seafood like shrimp lived at the bottom of sea, it can easily accumulate high concentration of arsenic in their body.

Shrimp pastes as one of the well-known processed food which use the shrimp as its main ingredients can easily get contaminated with the arsenic. By ingesting the shrimp paste with certain amount of arsenic concentration over a period of time, human have high risk to get effected through the consumption of shrimp paste in their daily intake. The exposure to arsenic either through inhalation, ingestion or absorption, it can make the human prone to get non-carcinogenic health effect or carcinogenic health effects. Several non-carcinogenic health effects include irritation of stomach and intestine, dermatitis, allergic and hypersensitivity. On the other hand,

the exposure to arsenic also can cause several kinds of cancer such as liver, skin, bladder, and lung cancer.



Heavy metal  
= Study variables

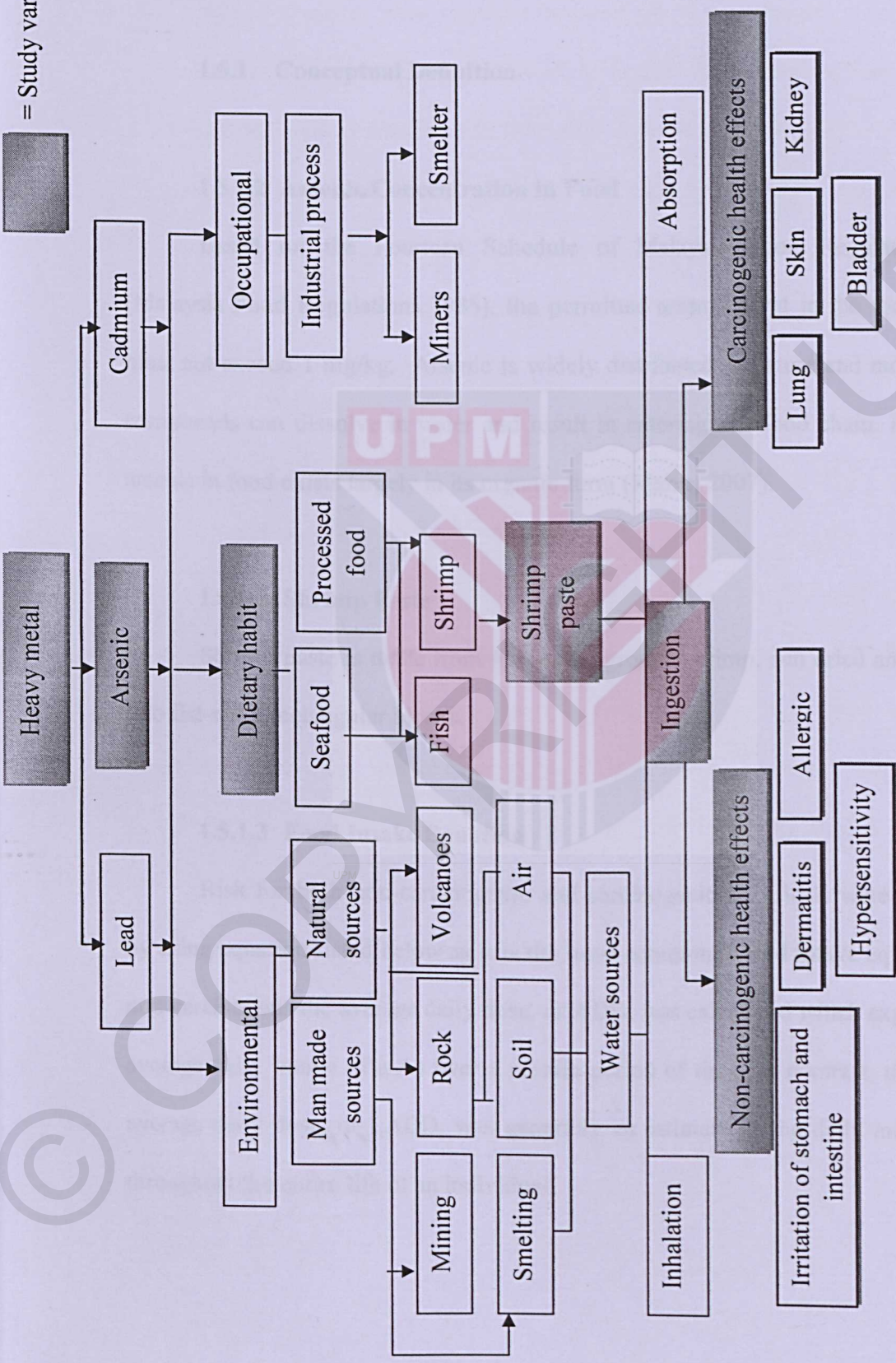


Figure 1.3: Conceptual framework

## 1.5 Definition of Terms

### 1.5.1 Conceptual Definition

#### 1.5.1.2 Arsenic Concentration in Food

Based on the Fourteen Schedule of Malaysia Food Regulation 1985 (Malaysia Food Regulations 1985), the permitted arsenic level in the food sample must not exceed 1 mg/kg. Arsenic is widely distributed in nature and most arsenic compounds can dissolve in water and result in entering our food chain. In general, arsenic in food exists largely in its organic form (Melve, 2007).

#### 1.5.1.2 Shrimp Paste

Shrimp paste is made from fermented ground shrimp, sun dried and then cut into fist-sized rectangular blocks.

#### 1.5.1.3 Food Intake Equation

Risk for both non-carcinogenic and carcinogenic chemicals were calculated by using equation listed below as it is the most commonly used intake equation. For non-carcinogens the average daily dose, or ADD, was calculated which expresses the average daily intake of a As over a certain period of time. In contrast, the lifetime average daily dose, or LADD, was generally an estimate of the daily intake of As throughout the entire life of an individual.

#### **1.5.1.4 Health Risk Assessment**

Risk assessment is a scientific process by which quantification potential environmental hazards to human health are achieved. This process utilizes the tools of science, statistics to identify and measure a hazard, determine possible routes of exposure, and finally use that information to calculate a numerical value to represent the potential risk. A human health risk assessment consists of four steps. These steps include hazard identification, dose-response assessment, exposure assessment, and risk characterization (Mallory, 2010).

#### **1.5.1.5 Arsenic Poisoning Symptoms**

Toxic effect caused by the ingestion or inhalation of arsenic or a substance containing arsenic, an ingredient in some pesticides, herbicides, dyes, and medicinal solutions. Small amounts absorbed over a period of time may result in chronic poisoning, producing nausea, headache, coloration and scaling of the skin, hyperkeratosis, anorexia, and white lines across the fingernails. Ingestion of large amounts of arsenic results in severe GI pain, diarrhea, vomiting, and swelling of the extremities. Renal failure and shock may occur, and death may result (Mosby's Medical Dictionary, 2009)

## 1.5.2 Operational Definition

### 1.5.2.1 Arsenic Concentration in Food

The concentration of arsenic in shrimp paste was quantified by using the Inductively coupled plasma mass spectrometry (ICP-MS). According to the Malaysia Food Regulation 1985, the level of total arsenic permitted in food items should not more than 1 mg/kg. The arsenic concentration in the sample of shrimp paste was expressed in  $\mu\text{g/L}$ (ppb). Then, the value was applied into the following equation to obtain the actual As concentration regardless the dilution factors.

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

Where,

A= concentration of arsenic in the extract ( $\mu\text{g/ml}$ ), B=Dilution factor, C= the volume of extract (ml), W= weight of sample (g).

### 1.5.2.2 Food Intake Equation

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).

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

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<sub>NC</sub>=Averaging time (EDx365days/yr)

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

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<sub>C</sub>=Averaging time (25,550 days/yr)

### 1.5.2.3 Health Risk Assessment

In this study, health risk assessment was defined and done specifically on the arsenic exposure through the shrimp paste consumption. The assessment was evaluated through the Hazard Quotient (HQ) for non-carcinogenic effects and Lifetime cancer risk (LCR) for carcinogenic effect to estimate the health risk of the respondents who exposed to arsenic through shrimp paste intake in their daily diet.

Non carcinogenic risks were quantified by the calculation of a Hazard Quotient (HQ) as below (III): The oral route was the route of exposure observed in this study; therefore, oral RfD values from the IRIS database was used.

$$\text{Hazard quotient (HQ)} = \frac{\text{ADD}}{\text{RfD}} \quad \text{(III)}$$

Where:

HQ = Non cancer Hazard Index of a health effect from intake of As, ADD = Average daily dose (mg/kg-day), Oral RfD = Oral Reference dose of arsenic (mg/kg-day) (0.0003 mg As/kg/day) (IRIS, 1998).

Then, the hazard quotient value was compared with the following values of risk acceptability for non-carcinogenic health effects. In cases where the non-cancer HQ does not exceed unity (HI < 1), it is assumed that no chronic risks are likely to occur at the site (Mallory, 2010).

**Table 1.1: The risk acceptability for non-carcinogenic health effect (U.S. EPA., 2007)**

Hazard Quotient (HQ)	
>1	Unacceptable
<1	Acceptable

As expressed earlier, carcinogens were assumed to not have an effective or safe threshold. Carcinogenic risk was expressed as cancer potency ( $q^*$ ) value and the following equation (IV) was used to quantify lifetime risk of cancer:

$$\text{Lifetime cancer risk (LCR)} = \text{LADD} \times q^* \tag{IV}$$

Where:

LADD = Lifetime average daily dose (mg/kg-day),  $q^*$  = Cancer potency factor, also known as slope factor (mg/kg-day), (1.5 mg/kg-day) (IRIS, 2007)

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.

**Table 1.2: The risk acceptability for carcinogenic health effects (USEPA., 2007)**

Lifetime cancer risk (LCR)	
$<10^{-6}$	Clearly acceptable
$10^{-6}$ to $10^{-4}$	Acceptable
$> 10^{-4}$	Clearly unacceptable

#### **1.5.2.4 Arsenic Poisoning Symptoms**

Symptoms associated with the acute arsenic poisoning indicated by the perception of respondent having the symptoms like vomiting, diarrhea, rashes, muscles cramp, hair loss, and stomach pain. For symptoms associated with chronic arsenic poisoning includes keratosis on the palms and soles, weight loss and numbness, and cancer.

### **1.6 Objective**

#### **1.6.1 General Objective**

To determine the arsenic concentration in shrimp paste and health risk among respondents in two villages in Melaka.

#### **1.6.2 Specific Objectives**

- 1) To determine the socio demographic data of respondents.
- 2) To determine the arsenic concentration in shrimp paste produced in Malacca.
- 3) To determine the shrimp paste frequency intake among respondents.
- 4) To determine the prevalence of signs associated with arsenic poisoning among respondents.

- 5) To determine the health risk of respondents from the consumption of shrimp paste indicates by Average Daily Dose (ADD), Lifetime Average Daily Dose (LADD), Hazard Quotient (HQ), and Lifetime Cancer Risk (LCR).
- 6) To determine the relationship between the shrimp paste frequency intake and health risk encountered by the respondents.
- 7) To determine the association between shrimp paste frequency intake and the prevalence of signs associated with arsenic poisoning among respondents.

## 1.7 Hypothesis

- 1) There is relationship between the shrimp paste frequency intake and health risk encountered by respondents.
- 2) There is association between shrimp paste frequency intake and the prevalence of signs associated with arsenic poisoning among respondents.

## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 Arsenic concentration in food

Diet plays a major role in cancer aetiology and prevention (AICR, 1997). Previous epidemiological studies, supported by preclinical data from animal and in vitro experiments and by clinical findings, have contributed immensely in providing insights into links between diet and cancer prevention and to the development of diet and cancer hypotheses (Sharif *et al.*, 2005). Carcinogens in the diet that trigger the initial stage of cancer include nitrosamines (in smoked and cured products), heavy metals, additives and preservatives (Reddy *et al.*, 2003).

Shrimp paste and salted fish contained the highest percentage of salt (sodium chloride) with 20% followed by dried shrimp with only 4%. However, total arsenic was found in each food item. The levels of total arsenic in each food item were much

higher compared to the level permitted by the Malaysian Food Act 1983 (1 mg/kg). Shrimp pastes contained the highest level of total arsenic with 6.16 mg/kg followed by dried shrimps with 4.03 mg/kg and salted fish which contained the least (1.89 mg/kg). The highest level of total ash was found in shrimp paste with  $34.26 \pm 2.79\%$  followed by salted fish with  $26.63 \pm 1.10\%$  and dried shrimps with  $12.43 \pm 0.15\%$ , respectively (Sharif *et al.*, 2005).

Furthermore, high levels of arsenic were found in all samples. This could be due to the ability of microorganisms in the environment to convert arsenic to dimethyl arsenate, which then accumulates in fish (Hodgson and Levi., 2000). Besides, arsenic may be a contaminant during processing and may also be from ground water that has been used in the processing of the product. The common regulation refers to total arsenic on the assumption that it would be mainly inorganic. The maximum permissible concentration of arsenic in food is currently 1.0 mg/kg. The daily intake of arsenic by humans reflects the quantity of seafood in the diet in which arsenic occurs mainly in the organic form (Sharif *et al.*, 2005).

## **2.2 Health effect from arsenic exposure**

Indeed, another point of genotoxic effect of arsenic is found when enzymes such as superoxide dismutase and catalase that scavenge for oxygen free radicals

seem to provide protection against arsenic-induced DNA damage (Mandal and Suzuki, 2002). The study revealed that arsenic may cause DNA damage by inhibiting DNA repair mechanisms where it can bind strongly to dithiols and sulfhydryl group. Such protein binding can induce inhibited DNA repair, mutation in key genetic sites, or increased cell proliferation, which could then lead to subsequent mutation via inhibited DNA repair.

Arsenic levels in marine organism are influenced by species differences, size of organism, and human activities. Bottom dwellers such as shrimp, crab, and lobster accumulate more arsenic than fish due to their frequent contact with bottom sediments (Attar *et al.*, 1992). Shrimp constitute approximately 30% of mean total seafood consumption in Kuwait (Khordagui and Al-Ajmi, 1991). Harvey *et al.*, (2002) reported that there is clearly a very serious problem of arsenic contamination in groundwater in much of southern and eastern Bangladesh

Since hand tube wells and shallow tubewells are the major water sources for safe drinking water in rural areas of Bangladesh, arsenic concentration higher than recommend values in these sources have been becoming serious health threat (Chen and Ahsan., 2004; Rahman *et al.*, 2006).

Several studies showed that the arsenic affected people in Bangladesh are 30 to 35 million (Jakariya *et al.*, 2007). Serious health hazards can be occurred due to arsenic contaminated drinking water use after a long period of about 5 to 15 years, but the duration can even be 2.5 years for high exposure of contamination (Harvey *et al.*, 2006). Slow arsenic poisoning observed in scalp hair samples among the As contaminated water consumers (Uddin *et al.*, 2006). These range from skin lesions (hyper-pigmentation, de-pigmentation, melanosis, keratosis, etc.) to cancers of the bladder, kidney, lungs and cardiovascular problems (Hossain, 2006).

Safiuddin and Karim (2001) reported that the melanosis (93.5 %) and keratosis (68.3 %) are the most common sufferings among the As affected people in Bangladesh. Thereafter, the seriously affected people are by arsenical (arsenite and arsenate), resulting skin cancer of about 0.8 % of the total skin disease patients. The scale of this environmental disaster is greater than any seen before. Chronic arsenicosis plays a crucial role in social and economic consequences as well as through victim's household economy, ultimately decreasing the quality of life (Safiuddin and Karim, 2001).

The accumulation of trace elements in environmental samples (soil, sediment, water, biota, etc.) can cause a potential risk to human health due to the transfer of these elements in aquatic media, their uptake by plants and subsequent introduction into the food chain. It is therefore necessary to monitor human exposure to toxic

trace elements present in the food chain, since number of studies have reported the total arsenic content of foodstuffs from different countries (Dabeka *et al.*, 1993; Tsuda *et al.*, 1995; Sapunar-Postruznik *et al.*, 1996; Roychowdhury *et al.*, 2002, 2003; Meharg and Mazibur, 2003; Alam *et al.*, 2003; Das *et al.*, 2004). Furthermore, according to Villa *et al.*, (2002), arsenic is ubiquitous in the environment, being naturally present in soil, air, water and food, and concentrations may be increased by anthropogenic contamination.

It is present in the environment in a number of different inorganic and organic chemical forms due to its participation in complex biological and chemical processes. Some of the most important arsenic species from a toxicological perspective include the two oxidation states As (III), As (V), mono-methyl arsonic acid (MMA), di-methyl arsinic acid (DMA), arsenobetaine and arsenocholine. Humans are exposed to many different inorganic and organic arsenic species present in food, water and other environmental media. Routes of arsenic intake include respiratory for dust and fumes and oral for water, beverages, soil and food. The most common mode of arsenic toxicity in humans is the inactivation of an enzyme system by binding through various biological ligands (Nagvi *et al.*, 1994).

Chronic exposure to inorganic arsenic may give rise to several health effects on the gastrointestinal tract, respiratory tract, skin, liver, cardiovascular system, hematopoietic system, nervous system, etc. Some of these human health effects are

currently being observed in populations in south and south eastern Asia, particularly in countries such as Bangladesh, India and Taiwan. Arsenic is recognized as a toxic element and has been classified as a human carcinogen to skin and lungs (WHO, 1980). According to Londesborough *et al.*, (1999), it has been reported that the toxicity of arsenic decreases with increasing methylation.

### 2.3 Health Risk Assessment

A human health risk assessment consists of four steps. These steps include hazard identification, dose-response assessment, exposure assessment, and risk characterization (California EPA, 2000).

#### 2.3.1 Hazard Identification

The first step, hazard identification, focuses on whether exposure to a particular chemical is capable of causing adverse health effects. A qualitative literature evaluation can show if exposure to a chemical causes an increase in the incidence of particular adverse health effects and whether those health effects occur in humans.

### **2.3.2 Dose-response Assessment**

Once adverse health effects are identified for a chemical, the dose-response assessment is used to quantitatively determine a relationship between the dose of the chemical and the incidence of adverse health effects in the exposed population. This step involves the evaluation of toxicity information in animals and humans and there are different models used to quantify dose-response based on whether the chemical is carcinogenic or non carcinogenic.

### **2.3.3 Exposure Assessment**

The third step in the risk assessment process is exposure assessment and this step estimates the amount and route by which a person is exposed to a chemical.

### **2.3.4 Risk Characterization**

Finally, the last step is risk characterization. This step brings together all the information gathered during the previous three steps to provide a quantitative estimate of the risk associated with exposure to the chemical of concern.

When conducting a human health risk assessment the EPA follows the risk assessment procedures developed by the National Academy of Sciences (1991). Chemicals of concern in a risk assessment fall into one of two categories, non

carcinogen or carcinogen, which determines the procedure for how the chemical is assessed and potential risks calculated. Non carcinogenic chemicals are assumed to have a threshold, a dose below which no adverse health effects will be observed. This no observed adverse effect level, or NOAEL, is a specific dose reported in laboratory animal studies to show no adverse health effects below the dose and to show adverse health effects above that dose (EPA, 1997).

Also part of the dose-response analysis is the value called the LOAEL, or the lowest observed adverse effect level. The LOAEL is the lowest dose of a chemical where an adverse response presents itself. An essential part of the dose-response portion of a risk assessment includes the use of a reference dose (RfD). The RfD is based upon toxicological data from animal studies and marks the highest average daily exposure over a lifetime that would not be expected to cause adverse human health effects (EPA, 2000).

Unlike non carcinogens, carcinogens are assumed to have no effective threshold. This assumption implies that there is a risk of cancer developing with exposures at low doses and, therefore, there is no safe threshold for exposure to carcinogenic chemicals. When evaluating a dose-response curve created from toxicological data the low doses of a chemical are assumed to follow a linear pattern and are therefore the data can be used to extrapolate from high doses to predict how populations will react at low doses. Carcinogens are expressed by their Cancer

Potency Factor (CPF) or  $q^*$ . This measure is derived from animal studies and uses the 95% confidence limit of the slope of the chemical's dose-response curve.

## CHAPTER 3

### METHODOLOGY



#### All Study Designs

This was a cross-sectional study conducted by arsenic exposure of residents of Malacca from 1997 to 2002. The study was conducted by the nearby Small Medical Clinic (SMC) in Malacca, which produced by the study could provide information of the exposure and the characteristics of risk factors associated with the specific point of time. However, the health risk as the outcome from the arsenic exposure through ingestion of drinking water could be estimated by taking into account the arsenic concentration in drinking water sample and the frequency intake could be assessed during the study at specific period of time. The study conducted from January 2012 until February 2012.

## CHAPTER 3

### METHODOLOGY

#### 3.1 Study Design

This was a cross sectional study which focused on arsenic exposure of residents of Malacca from the shrimp paste consumption which produced by the nearby Small Medium Industry (SMI) and home-made shrimp paste. This type of study could provide a 'snapshot' of the outcome and the characteristics or risk factors associated with it, at a specific point in time. Hence, the health risk as the outcome from the arsenic exposure through ingestion of shrimp paste could be estimated by taking into account the arsenic concentration in shrimp paste sample and respondents' frequency intake could be assessed simultaneously at specific period of time. This study commenced from January 2012 until February 2012.

### 3.2 Study Location

This study was conducted at Kg. Pinang (Dun Klebang) and Kg Pantai Rombang (Dun Pantai Kundur), in the state of Malacca which located nearby the shrimp paste production area in the district of Melaka Tengah, Malacca (Appendix 1I: Figure 3.1). For that reason, it was assumed that the population of both villages got their main source of shrimp paste from the nearest industry. Hence, these 2 villages were chosen as the location of study to be conducted.

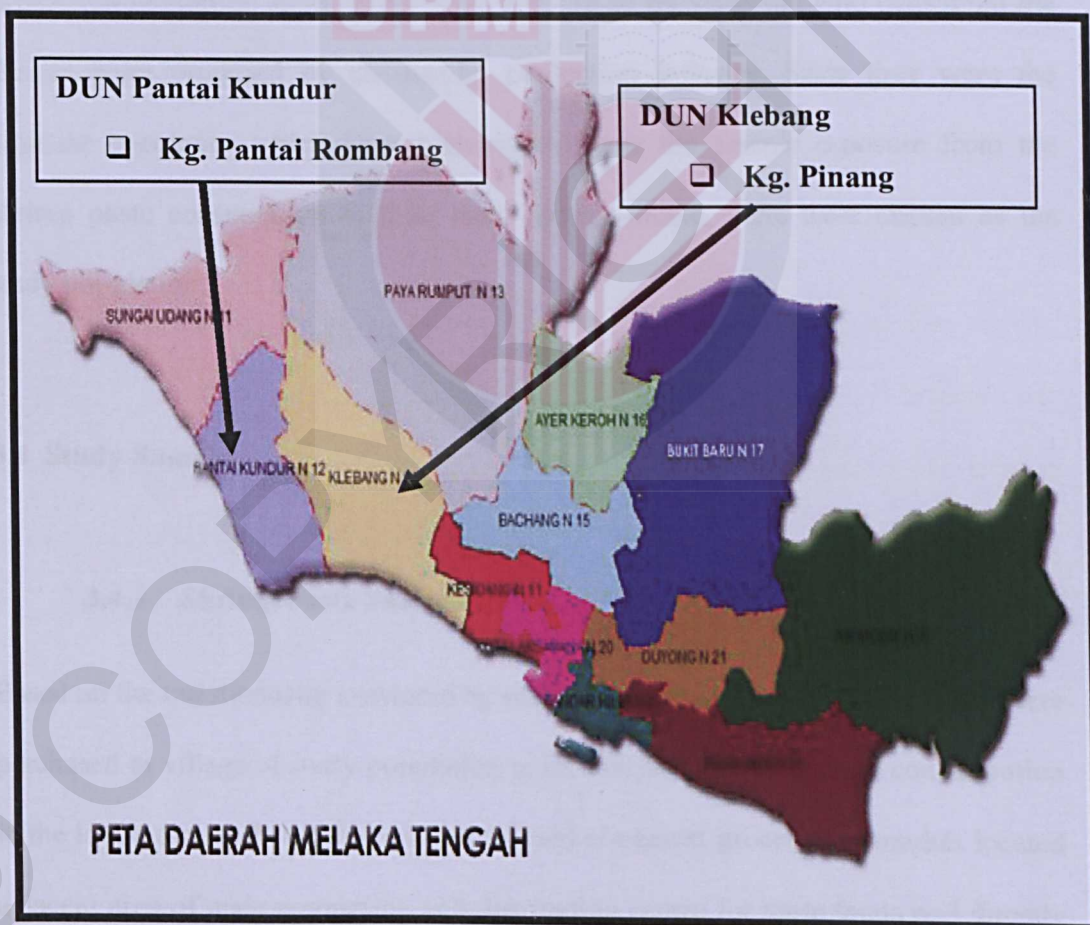


Figure 3.2: Location of study areas

(Source: Pejabat Tanah Daerah Melaka Tengah, 2011)

### **3.3 Study Population**

The residents of Kg. Pinang and Kg Pantai Rombang which resided nearest to the areas where the shrimp paste were produced were included in the study population. Indeed, there were approximately 1001 residents lived in the Kg. Pinang and 1005 residents in Kg. Pantai Rombang which the study's respondents were selected from. Malay was the main race settled in these 2 villages followed by the Chinese and India. Besides, there were 5 persons in each house. The study population comprised adult respondents aged 18 to 64 years old who consumed the shrimp paste produced by the nearby production industry. Since they were the adjacent population which have high risk to have the arsenic exposure from the shrimp paste consumption in their daily dietary intake, there were chosen as the study population.

### **3.4 Study Sample**

#### **3.4.1 Shrimp Paste Sample**

Based on the questionnaire answered by respondents, 5 samples of shrimp paste were purchased at village of study population to be analysed for the arsenic concentration in the laboratory. All samples were purchased at nearest groceries or market located adjacent area of main production and distribution centre for these foods and directly

from the villagers. Then, the shrimp paste samples were analyzed by using the ICP-MS in order to measure the arsenic concentration.

### 3.4.2 Respondents

The respondents were recruited from the 2 villages included Kg. Pinang and Kg. Pantai Rombang. All these villages located nearest to the main production area of the shrimp paste. Indeed, the respondents were randomly chosen. The respondents were adult villagers from 18 to 64 years old. Apart from that, the respondents must be the consumer of local shrimp paste as part of their dietary intake.

### 3.5 Sample Size

For sample size, 10% of total population is recruited as sample (Neuman et al., 1997). According to Neuman *et al.*, (1997) if the total population is between the range of 1000 to 10000, 10% is sufficient to represent the total population. Hence, this sample size of this study was as follows:

a) Kg. Pinang: Total population = 1010 residents

$$= \frac{10}{100} \times 1010$$
$$= 101 \text{ respondents}$$

b) Kg. Pantai Rombang: Total population = 1050

$$= \frac{10}{100} \times 1050$$

$$= 105 \text{ respondents}$$

Total respondents: 206 respondents

The response rate of this study was 73% (151 respondents) which did not reach the actual amount of respondents as the calculation of sample size (206 respondents). It was due to the time constraint, the respondents refused to take part in the study and unavailability of villagers during study was conducting. However, according to Groves and Robert (2006), a working assumption for a survey to be construed as "good," it must attain a high response rate (e.g., 70 percent). Hence, 73 percent of response rate could represent the total studied population.

### 3.6 Sampling Method

#### 3.6.1 Food Sample

Total of 5 different shrimp paste samples were purchased in the shops and directly from villagers which located at the same villages in which the respondents were recruited. Then, the samples were brought to the laboratory for the analyzing of arsenic concentration.

Then, shrimp paste samples were analyzed by using the ICP-MS. Plasma MS is a widely recognized technique for trace element analysis and being increasingly used as a detector for chromatographic determinations. The benefits of coupling plasma mass spectrometric detection with chromatographic separation include element specificity, real-time chromatograms, and the ability to separate interferences from peaks of interest, multi-element capability and low levels of detection (sub-nanogram for most elements). These features of ICP-MS were valuable assets in the speciation of trace elements, where sample pre-treatment should be minimized. Sample pre-treatment and preconcentration can lead to changes in the relative concentration of individual species. Ion chromatography (IC) is an attractive analytical technique for element speciation because it can separate both inorganic and organic charged species in addition to free ions.

### **3.6.2 Respondents**

At first, residents of Kg. Pinang and Kg. Pantai Rombang were purposely chosen to become the study population of this research as the locations were main production area of shrimp paste in the state of Malacca. Then, the study sample in the age of 18 to 64 years old, and local-production shrimp paste's consumer were randomly chosen to be included in this study.

### 3.7 Instrumentations and Data Collection

#### 3.7.1 Questionnaires

In this study, the questionnaire was utilized in order to assess the respondents' basic information, and the frequency intake of shrimp paste. The questionnaire included 5 sections consisted as following (Appendix I).

- I. Section 1: Socio-demography. This form contained questions on the respondents' identification number (ID), socio-demographic data i.e. sex, ethnicity, religion, marital status, date of birth, educational level, occupation, individual income and family income.
- II. Section 2: Health Status. This section consists of questions on respondents' health status. They were asked regarding the symptoms which might be persisted by them.
- III. Section 3: Anthropometry This form contained information about the respondents, i.e, weight and height. There were spaces for three measurements of weight and height. The measurement for weight was in kilogram and height in centimetres.
- IV. Section 4: Frequency of Food Intake Information
- V. Section 5: Others Possible Sources of Arsenic Exposure. This section consisted of 3 items which might give other sources of arsenic exposure to the respondents. It included sources of drinking water, smoking habit and alcohol consumption.

This section was universally known as the Food Frequency Questionnaire (FFQ). The form had listed 41 food items which were categorised into 6 food groups. According to the Zukowska on Methodological Evaluation of Method for Dietary Heavy Metal Intake, a food frequency questionnaire (FFQ) method was utilized as a tool in assessing the frequency of individual foods or food group's intake over extended periods of time (weeks, months, or years). For that reason, the food frequency questionnaire was chosen as one of study instrumentation. The underlying principle of this approach was the possibility of a long-term evaluation of shrimp paste intake, which might provide more valuable information for estimating average exposure to arsenic than short-term methods.

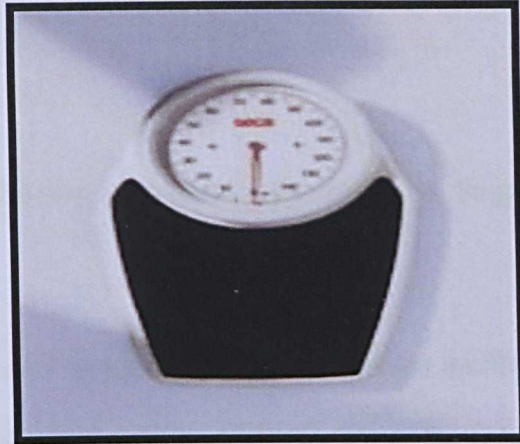
The FFQ enabled the obtainment of useful information on the consumption of shrimp paste containing arsenic and the quantity of its consumption. Besides, the FFQ consisted of a structured list of other kind of food and a frequency of its consumption by respondents. There were four main columns in the FFQ. The first column contained list of food items while the second column described the frequency of intake by day, week, month, year or not eaten at all. The frequency of intake was based on the habitual food intake during the past year. The third column described the serving size of each food item while the fourth column described the number of servings consumed each time the food item is eaten.

### 3.7.2.1 Arsenic Concentration in Shrimp Paste Sample

Arsenic concentration was measured by using the Perkin-Elmer Model ELAN-DRC Inductively coupled plasma mass spectrometry (ICP-MS) (Figure 3.2). With sufficient sensitivity of ICP-MS, an accurate determination of arsenic concentration in shrimp paste could be measured. It is because the heavy metals for which dietary exposure is of interest are present in trace and ultra-trace quantities. The benefits of coupling plasma mass spectrometric detection with chromatographic separation include element specificity, real-time chromatograms, the ability to separate interferences from peaks of interest, multi-element capability and low levels of detection (sub-nanogram for most elements) (Brenda and Joseph, 1992).

### 3.7.3 Seca Digital Platform Scales (Model 880)

*Seca* digital platform scale (Figure 3.4) was used to measure weight of respondents. The scales were able to measure up to 200 kilograms. The scale was placed on a hard and level surface when weighing the respondent.



**Figure 3.4: *Seca* digital platform scales (Model 880) which was used to measure the weight of respondents.**

#### **3.7.4 Portable *Seca* Body Meter**

A portable *Seca* body meter (Figure 3.5) taped to a vertical wall and perpendicular to a level floor was used for height measurements. The tape was graded in centimetre with one millimetre divisions. This portable body meter was very light (200 gm), small and able to measure up to 200 centimetres.



**Figure 3.5: Portable *Seca* body meter which was used to measure height of respondents**

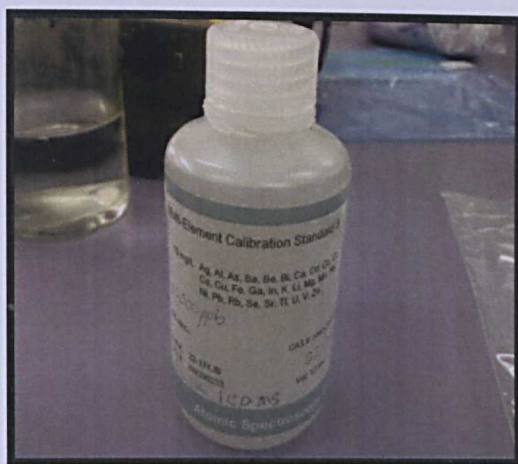
## 3.8 Sample Analysis

### 3.8.1 Dry Ashing Method for Shrimp Paste Sample Preparation for ICP-MS.

One gram of sample in a crucible was placed in a preheated muffle furnace at 200–250 °C for 30 min, and then ashed for 4 h at 480 °C. Then, the sample was removed from the furnace and cooled down; 2 ml of 5 M HNO<sub>3</sub> was added and evaporated to dryness on a water bath. Next, the sample was placed in a cool furnace and heated to 400 °C for 15 min, before being removed (from the furnace, cooled and moistened with four drops of distilled water). Next, 2 ml of concentrated HCl was added and the sample was evaporated to dryness, removed, and then 5 ml of 2 M HCl was added and the tube was again swirled. The solution was filtered through Whatman No. 42 filter paper and <0.45 μm Millipore filter paper, and then transferred quantitatively to a 25 ml volumetric flask by adding distilled water (Issac and Kerber, 1971) (Appendix III).

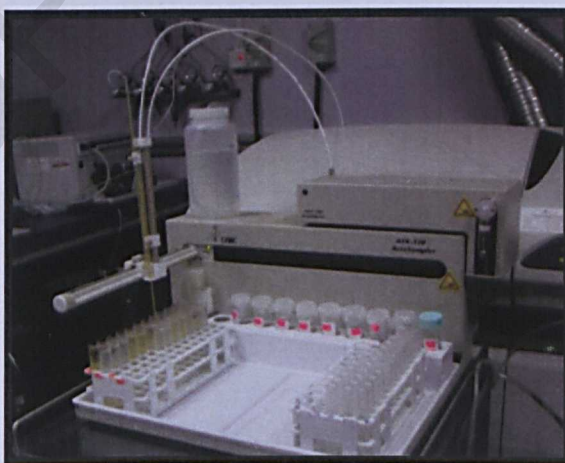
### 3.8.2 Inductively coupled plasma mass spectrometry (ICP-MS)

For the sample analysis, the ICP-MS Model ELAN DRC Perkin Elmer was used in detection of As. A minimum sample volume of 10-20 ml was needed for the analysis, however, it was essential that the sample is representative, and extreme care must be taken to avoid contamination of the sample.



**Figure 3.12: As Standard solution**

At first, the standard solution (Figure 3.12) of 10 000ppb was prepared. It was diluted into 10 ppb, 30 ppb, 50 ppb and 100 ppb. Next, all the samples were labeled properly and poured into the cuvette tube and placed in the rack before it were analyzed by ICP-MS. Then, in the same rack, 4 prepared standard solutions (10 ppb, 30 ppb, 50 ppb and 100 ppb) and one blank sample were placed together to be analyzed in order to get the standard curve of samples.



**Figure 3.13: Sample analyzation by ICP-MS**

First of all, the ICP-MS (Figure 3.13) would analyze the blank sample and 4 standard solutions in order to obtain the standard curve for samples. When the standard curve give the value of  $\pm 0.9999$  (linear through zero), it indicated that the prepared standard solutions were accepted to be used during analyze of sample.

The process of ICP-MS in analysing the sample includes the introduction of liquid sample into argon-based and high-temperature radio frequency plasma. Then, ions generated are extracted from the plasma and separated on the basis of their mass-to-charge ratio by a mass spectrometer. The technique is useful for multi-element determination and solid samples and samples containing precipitates must be digested prior to analysis.

In general, detection limits of 0.02 and 1  $\mu\text{g l}^{-1}$  are quoted. However, when the procedure includes sample digestion detection limits would be expected to be higher (Rasmussen *et al.*, 2004). Each shrimp paste sample was duplicated 2 times and ready for ICP-MS analysis. The averages of the concentrations was then taken and compared with the permitted maximum level of arsenic in food stated in Seventh Schedule of Malaysia Food Regulation 1985 (Malaysia Food Act 1983).

### **3.9 Quality Control**

#### **3.9.1 Standard Operating Procedure (SOP)**

In order to prevent any analytical error while analyzing the arsenic concentration in shrimp paste, all the preparation and procedures was conducted according to the guideline for The Perkin-Elmer ICP-MS provided by the manufacturer. Furthermore, the weighing scales and height measuring instrument used in the survey were standardized and calibrated to ensure the validity of the measurement.

#### **3.9.2 Instrument Calibration**

The ICP-MS would be performed the daily performance report in order to determine the level of instrument performance. Some correction would be done if the instrument showed the abnormal current optimization file data.

#### **3.9.3 Pre Test of Questionnaire**

The pre-test questionnaires were performed on 10% of sample size before the start of the study on selected adults to ensure the understanding of the questions. The questionnaire was tested on a random sample of the community to assess its comprehensiveness, and clarity of questionnaire. This test was conducted to the residents of Tg. Sedili, Johor. A total of 21 respondents were recruited which was 10% of total study population.

### 3.10 Data Analysis

All data was analyzed with SPSS 19.0 Evaluation Version Product. Descriptive analysis was used to describe the data on socio demographic information. The statistical test employed was Spearman's rho to determine relationship between shrimp paste frequency intake and LCR and HQ. The chi-square test was utilized in order to determine association between the shrimp paste frequency intake and As acute and chronic poisoning signs. Statistical significance was defined as  $p < 0.05$ .

### 3.11 Ethical Issues

The study protocol was reviewed and approved by the Medical Research Ethical Committee, Faculty of Medical and Health Sciences, Universiti Putra Malaysia (Appendix I). The written consent was signed by respondent before the commencement of data collection. All respondents were gave a choice to continue participating in the study or to pull out at any time they choose to do so. Finally, all the information about the respondents' background involved in this research remained in confidential.

Table 4.1: Socio-demographic data of respondents (N=151)

Variable	N	%	Med (IQR)	Mean±SD	Range
<b>Age (years) :</b>					
18-29	34	23	44(24)	45.12±6.1	18-64
30-49	36	24			
50-64	81	53			
<b>Gender :</b>					
Male	36	24			
Female	95	63			
<b>Ethnic :</b>					
Malay	150	99			
Chinese	1	1			
<b>Income per month (RM) :</b>					
< 720	1	1			100-3600
720-1440	28	19			
1440-2160	28	19			
2160-2880	28	19			
2880-3600	66	44			
<b>Household income (RM) :</b>					
< 720	28	19			200-7000
720-1440	28	19			
1440-2160	28	19			
2160-2880	28	19			
2880-3600	66	44			

## CHAPTER 4

### RESULTS

#### 4.1 Socio-demographic information of respondents

Table 4.1 shows the demographic characteristics of the subjects recruited into the study. All the respondents were recruited from 2 villages in Melaka namely Kampung Pantai Rombang and Kampung Pinang. The proportion of female was higher compare to male which were 2:1. The majority of respondents were Malay who falls into high household income category. With regard to age, about 77% of respondents were more than 30 years old and 40% of them were under range of 50 to 64 years old. Besides, majority of household consisted of 3 to 7 person per household. About 48.3% of respondents were in normal range of Body Mass Index which was 18.5 – 24.9kg/m<sup>2</sup>.

**Table 4.1: Socio-demographic data of respondents (N=151)**

<b>Variable</b>	<b>N</b>	<b>%</b>	<b>Med (IQR)</b>	<b>Mean±SD</b>	<b>Range</b>
<b>Age (years) :</b>					
18-29	34	23	44(24)	43±12.64	18-64
30 – 49	56	37			
50-64	61	40			
<b>Gender :</b>					
Male	56	37.1			
Female	95	62.9			
<b>Race:</b>					
Malay	150	99.3			
Chinese	1	0.7			
<b>Income per month(RM):</b>					
< 720	53	35.1	800(500)	923±603	200-3600
≥ 720	98	64.9			
<b>Household income(RM):</b>					
<720	43	28.5	900(600)	1044±711.99	200-7000
≥ 720	108	71.5			
<b>Household:</b>					
<3	19	12.6	5(3)	5±0.24	0-10
3-7	124	82.1			
≥8	8	5.3			
<b>Height(cm) :</b>					
			160(15)	161±8.68	138-180
<b>Weight (kg):</b>					
			66(16)	66±13.14	32-115
<b>BMI (kg/m<sup>2</sup>) :</b>					
<18.5(underweight)	3	2	24.9(6.34)	25.5± 5.09	13.5-45.3
18.5 – 24.9(normal)	73	48.3			
25-29.9 (pre-obese)	51	33.8			
30≥ (obese)	24	15.9			

## 4.2 Shrimp paste frequency intake of respondents

Figure 4.1 shows the shrimp paste frequency intake of respondents. It exhibits that about 39 % of respondents consumed 2 to 6 times a week and 38% consumed shrimp paste as their daily diet intake. The rest of respondents had consumed once a week (5.3%), 2 to 3 times a month (5.9%), once a month (5.3%), and seldom (5.3%). Table 4.2 shows the mean value of ADD and LADD of respondents. The mean value of ADD was  $1.4 \times 10^{-6} \pm 4 \times 10^{-7}$  and  $7 \times 10^{-7} \pm 3 \times 10^{-7}$  for LADD (mg/kg/day).

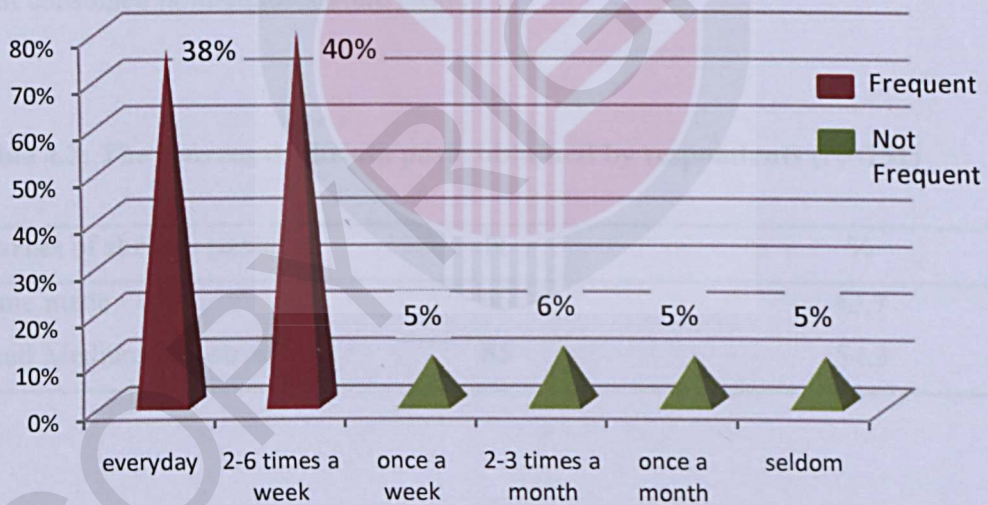


Figure 4.1: Frequency intake of shrimp paste among respondent (N= 151)

**Table 4.2: Mean of Average Daily Dose and Lifetime Average daily dose of respondents. (N=151)**

<b>ADD(mg/kg/day)</b>	<b>LADD(mg/kg/day)</b>
<b>Mean±SD</b>	<b>Mean±SD</b>
$1.4 \times 10^{-6} \pm 4 \times 10^{-7}$	$7 \times 10^{-7} \pm 3 \times 10^{-7}$

Table 4.3 shows the sources of shrimp paste obtained by respondents. Respondents gained the shrimp paste supply either homemade shrimp paste or purchased from the nearest small medium industries. About 54.3% of respondents purchased the shrimp paste from the nearest Small Medium Industries and 43.7% of them consumed homemade shrimp paste.

**Table 4.3: The sources of shrimp paste obtained by respondents (N=151)**

<b>Sources of shrimp paste</b>	<b>N</b>	<b>%</b>
Home made	66	43.7
Small Medium Industries	85	54.3

### **4.3 Prevalence of acute and chronic of arsenic poisoning signs**

Table 4.4 shows the prevalence of acute and chronic arsenic poisoning signs among the respondents. According to the result, majority of the respondents stated that they were not experienced any acute arsenic poisoning symptoms. The most 4 with higher prevalence among the respondents were dizziness (32.5%), lethargy

(24.5%), allergic (21.2%), and muscle cramp (15.9%). Furthermore, most of the respondents reported that they did not experience any chronic arsenic poisoning. However, the most 3 symptoms which show the highest prevalence were numbness (27.2%), hyperkeratosis (16.6%), and brittle nail (13.2%).

**Table 4.4: Prevalence of possible acute & chronic arsenic poisoning signs or health related problems**

Chronic	Yes		No	
	N	%	N	%
Thickness of palm and feet skin (hyperkeratosis)	25	16.6	126	83.4
Spots on skin	12	7.9	139	92.1
Brittle nail	20	13.2	131	86.8
Numbness	41	27.2	110	72.8
Cancer	7	4.6	144	95.5
Acute	Yes		No	
	N	%	N	%
Stomach ache	14	9.3	137	90.7
Vomiting	2	1.3	149	98.7
Diarrhea	5	3.3	146	96.7
Muscle cramp	24	15.9	127	84.1
Dizziness	49	32.5	102	67.5
Breathing difficulty	13	8.6	138	91.4
Lethargy	37	24.5	114	75.5
Allergic	32	21.2	11	78.8

#### 4.4 Other possible exposures to arsenic

There are other sources of arsenic exposure other than through food contamination. It includes contamination in drinking water, smoking habit and alcohol consumption. Table 4.5 shows the water sources utilized by respondents. Majority (98%) of them used the tap water in their daily activities and only 2% of them used well water. Furthermore, there were only 22.5% of respondents were smoking and 4.6% of them were already quit and only 0.7 % of respondents consumed alcohol.

**Table 4.5: Other possible exposure to arsenic**

Variables	N	%
<b>Water sources</b>		
Tap water	148	98.0
Well water	3	2.0
<b>Smoking Habit</b>		
Yes	34	22.5
No	109	72.2
Already quit	7	4.6
<b>Alcohol consumption</b>		
Yes	1	0.7
No	150	99.3

Table 4.6 and 4.7 show the prevalence and mean frequency of the top ten daily and weekly consumed foods among respondents. Generally 99% of respondents

consumed rice twice a day and on average 2 scoops was consumed. This result is expected as rice is the staple food of Malaysians. The other nine food items eaten daily but by a smaller proportion of the population ranged from rice, sugar, leafy vegetable, marine fish, cabbage, bitter/pumpkin/cucumber, nut vegetables, root vegetables, local salads (*ulam*) and baby corn. All these latter food items were consumed at least twice to once a day, in amounts ranging from two scoop of rice to one pieces of baby corn.

**Table 4.6: Prevalence and mean of top 10 daily consumed foods.**

Types of food	Prevalence (%)	Mean frequency per day	Total amount consumed per intake
Rice	99	1.93	2 scoop
Sugar	97	1.64	1 teaspoon
Leafy vegetable	84	1.46	1 cup
Marine fish	72	1.21	1 piece
Cabbage	68	1.13	1 cup
Bitter/pumpkin/cucumber	66	1.11	1 teaspoon
Nut vegetables	60	1.04	1 tablespoon
Root vegetables	60	1.04	1 tablespoon
Local salads ( <i>Ulam</i> )	54	0.95	1 teaspoon
Baby corn	52	0.89	1piece

As Malaysia has an abundance of varieties of foods, the results showed that the majority of Malaysians consume food items more regularly at weekly intervals. Table 4.8 shows the prevalence and mean frequency of the top ten weekly consumed foods by respondents. The food items range from the chilly/tomato sauce, light soy sauce, anchovies, nuts, peanuts, fish, soys bean cake (*Tempe*), soya bean curd (*tauhu*), oyster sauce, and cabbage. Mean frequencies ranged between 2.60 to 1.24 times per week. Total amount of food consumed per intake was ranged from 2 tablespoon of chilli/tomato sauce to 1 bowl of cabbage.

**Table 4.7: Prevalence and mean of top 10 weekly consumed foods**

Types of food	Prevalence (%)	Mean frequency per weekly	Total amount consumed per intake
Chilly/tomato sauce	56	2.60	2 tablespoon
Light soy sauce	51	2.64	2 tablespoon
Anchovies	42	4.52	2 tablespoon
Nuts	31	1.21	2 tablespoon
Peanuts	31	0.65	1 tablepoons
Fish	27	0.65	1 piece
Tempe	25	2.03	2 slices
Soya bean curd( <i>Tauhu</i> )	25	1.40	1 slice
Oyster sauce	23	1.26	1 teaspoon
Cabbage	20	1.24	1bowl

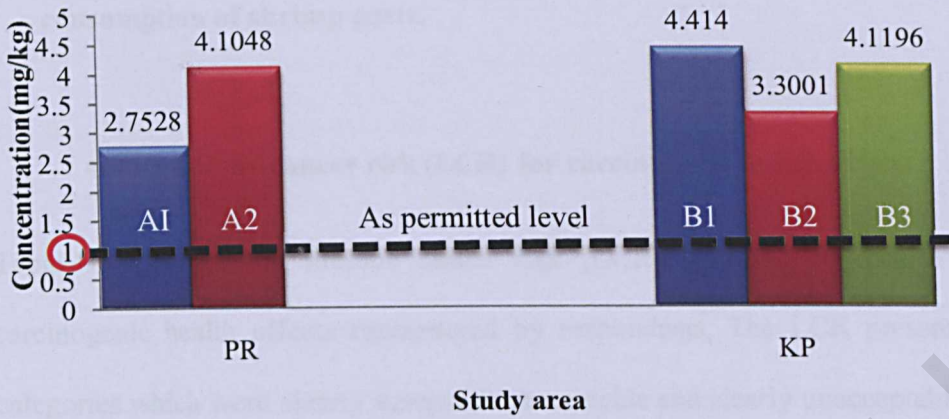
#### 4.5 Arsenic concentration in shrimp paste

Figure 4.2 shows the arsenic concentration detected in five samples of shrimp paste produced in Melaka. Two shrimp paste samples were purchased in Kg. Pantai Rombang and three samples in Kg. Pinang. The samples were analyzed using ICP-MS after being digested with dry ashing method. Besides, all samples were duplicated and reported in  $\mu\text{g/L}$ . Table 4.8 shows the parameters used in equation A in order to convert raw data from reading of ICP-MS to actual reading As concentration. After the conversion, the mean of arsenic concentration in sample 1(A1) sample 2(A2) of Kg Pantai Rombang were 2.7528 mg/kg, and 4.1048 mg/kg respectively as shown in figure 4.2. Furthermore, for Kg.Pinang, the mean of arsenic concentration in sample 1(B1), sample 2(B2) and sample 3(B3) were 4.414 mg/kg, 3.3001mg/kg and 4.1196 mg/kg respectively.

**Table 4.8: Parameters used in equation A to convert raw data of ICP-MS to actual reading of As concentration**

Parameter	Symbol	Unit	Parameter characteristics				
Concentration of arsenic in the extract	A	$\mu\text{g/ml}$	S*(A1)	S*(A2)	S*(B1)	S*(B2)	S*(B3)
Dilution factor	B	-	0.1147	0.171	0.184	0.138	0.172
Volume of extract	C	ml	4	4	4	4	4
Weight of sample	W	g	60	60	60	60	60

S\*= Sample



\*PR:Kg.Pantai Rombang\*KP:Kg. Pinang

**Figure 4.2: Arsenic concentration in shrimp paste samples**

Table 4.9 shows the mean concentration of As in shrimp paste samples in PR and KP. The mean As concentration for PR was 3.43mg/kg  $\pm$ 0.955 and 3.94mg/kg  $\pm$ 0.577 for KP. It was ranged from 2.75mg/kg to 4.10 mg/kg for PR and 3.30mg/kg to 4.41mg/kg for KP.

**Table 4.9: Mean concentration of Arsenic in shrimp paste sample**

Variable	PR		KP	
	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range
<b>Arsenic concentration(mg/kg)</b>	3.43 $\pm$ 0.955	2.75-4.10	3.94 $\pm$ 0.577	3.30-4.41

\*PR:Kg.Pantai Rombang\*KP: Kg.Pinang

#### 4.6 Health risk encountered by respondents from arsenic exposure through consumption of shrimp paste.

##### 4.6.1 Lifetime cancer risk (LCR) for carcinogenic health effects.

Table 4.10 shows the lifetime cancer risk (LCR) indicated for health risk of carcinogenic health effects encountered by respondents. The LCR presented in 3 categories which were clearly acceptable, acceptable and clearly unacceptable. There were 50% of respondents had clearly acceptable risk, 50% falls under acceptable risk and there was no respondents faced clearly unacceptable risk. For clearly acceptable category, the mean was  $7 \times 10^{-7}$  and ranged from  $2.4 \times 10^{-7}$ - $10 \times 10^{-7}$ . Indeed, for acceptable risk, the mean was  $1.5 \times 10^{-6}$  which ranged from  $1 \times 10^{-6}$ - $0.2 \times 10^{-5}$ .

**Table 4.10: Health risk for carcinogenic health effects**

LCR		Frequency	%	Mean±SD	Range
$<10^{-6}$	Clearly acceptable	88	58	$7 \times 10^{-7} \pm 2.4 \times 10^{-7}$	$2.4 \times 10^{-7}$ - $10 \times 10^{-7}$
$10^{-6}$ - $10^{-4}$	Acceptable	63	42	$1.5 \times 10^{-6} \pm 0.3 \times 10^{-6}$	$1 \times 10^{-6}$ - $0.2 \times 10^{-5}$
$>10^{-4}$	Clearly unacceptable	0	0	0	0

#### 4.6.2 Health quotient (HQ) for non-carcinogenic health effects.

Table 4.11 shows the health quotient indicated for health risk of non-carcinogenic health effects encountered by respondents. The HQ presented in 2 categories which were acceptable and clearly unacceptable. Based on the result, all the respondents had acceptable risk for non-carcinogenic health effects. The mean value for HQ was 0.0046 which ranged from 0.0034 to 0.0061

**Table 4.11: Health risk for non-carcinogenic health effects.**

HQ		Frequency	%	Mean±SD	Range
<1	Acceptable	151	100	0.0046±0.0013	0.0034- 0.0061
>1	Unacceptable	0	0		

#### 4.7 Relationship between shrimp paste frequency intake and health risk of respondents

##### 4.7.1 Relationship between frequency intake of shrimp paste and lifetime cancer risk (LCR) for carcinogenic health effect of respondents

The LCR for each respondent were calculated using equation IV. Table 4.12 shows the relationship between frequencies intake of shrimp paste and LCR for carcinogenic health effects which were analyzed using a Spearman's rho test. Based on the result, there was no significant relationship between frequency intake of shrimp paste and LCR of respondents since  $p$  value was more than 0.05.

**Table 4.12: Spearman's rho test between shrimp paste frequency intake and health risk (LCR) for carcinogenic health effects.**

Variables	LCR	
	<i>r</i>	<i>p</i>
Frequency intake of shrimp paste	0.088	0.281

\*significant at  $p < 0.05$

#### 4.7.2 Relationship between frequency intake of shrimp paste and hazard quotient (HQ) for non-carcinogenic health effect of respondents

The HQ for each respondent was calculated using equation III. Table 4.13 shows the relationship between frequencies intake of shrimp paste and HQ for non-carcinogenic health effects which were analyzed using a Spearman's rho test. Based on the result, there was no significant relationship in between frequency intake of shrimp paste and HQ of respondents since  $p$  value was more than 0.05.

**Table 4.13: Spearman rho test between shrimp paste frequency intake and health risk (HQ) for non-carcinogenic health effects.**

Variables	HQ	
	<i>r</i>	<i>p</i>
Frequency intake of shrimp paste	0.020	0.803

\*significant at  $p < 0.05$

#### 4.8 Association between shrimp paste frequency intake and possible signs of acute and chronic arsenic poisoning among respondents.

Table 4.14 shows the association between shrimp paste frequency intake and possible signs of chronic arsenic poisoning among respondents. Based on the result, there were no significant association between shrimp paste frequency intake and the prevalence of possible acute arsenic poisoning signs since  $p$  value was more than 0.05. Table 4.15 shows the association between shrimp paste frequency intake and possible signs of acute arsenic poisoning among respondents. However, there were no significant association between shrimp paste frequency intake and possible signs of acute arsenic poisoning among respondents since  $p$  value for each symptoms more than 0.05 except for diarrhea which  $p$  value was  $<0.05$ .

**Table 4.14: Chi-square test between shrimp paste frequency intake and possible signs of chronic arsenic poisoning among respondents**

Variables		Frequency intake of shrimp paste (%)		X <sup>2</sup>	p
Chronic		Frequent (n=118)	Less frequent (n=33)		
Thickness of skin palm and feet (hyperkeratosis)	Yes	16(14)	9(27)	3.510	0.061
	No	102(86)	24(73)		
Spots on skin	Yes	8(7)	4(12)	1.006	0.316
	No	110(93)	29(88)		
Brittle nail	Yes	15(13)	5(15)	0.134	0.715
	No	103(87)	28(85)		
Numbness	Yes	31 (26)	10(30)	0.212	0.645
	No	87(74)	23(70)		
Cancer	Yes	4(3)	3(9)	1.896	0.169
	No	114(97)	30(91)		

\*significant at p<0.05

**Table 4.15: Chi-square test between shrimp paste frequency intake and possible signs of acute arsenic poisoning among respondents**

Variables	Frequency intake of shrimp paste (%)		X <sup>2</sup>	P	
	Frequent (n=118)	Less frequent (n=33)			
Stomach ache	Yes	11(9.3)	3(9)	0.002	0.968
	No	107(91)	30(91)		
Vomiting	Yes	2(2)	0(0)	0.567	0.452
	No	116 (98)	33(100)		
Diarrhea	Yes	2(2)	3(9)	4.406	0.036*
	No	116(98)	30(91)		
Muscle cramp	Yes	21(18)	3(9)	1.462	0.227
	No	97(82)	30(91)		
Dizziness	Yes	37(31)	12(36)	0.295	0.587
	No	81(69)	21(64)		
Breathing difficulty	Yes	8(7)	5(15)	2.297	0.130
	No	110(93)	28(85)		
Lethargy	Yes	28(24)	9(27)	0.175	0.676
	No	90(76)	24(73)		
Allergic	Yes	27(25)	5(15)	0.923	0.337
	No	91(60)	28(85)		

\*significant at p<0.05

## CHAPTER 5

### DISCUSSION

#### 5.1 Discussion

##### 5.1.1 Socio-demographic of respondents

This study was conducted among 151 residents of Kg. Pantai Rombang and Kg. Pinang located in district of Melaka Tengah, Melaka. The socio-demographic data of respondents was very important to access the characteristic of respondents being studied. Majority of the respondents' age was 30 to 49 (37%), and 50 to 64 (40%).

According to the Oakland County Michigan Health Division, Department of Health & Human Services stated that elderly, people with long-term illnesses, and unborn babies are at greatest risk to As absorption. They can be more sensitive to chemical exposures. In general, the prevalence of arsenism increases along with

increasing of age and extended years of residence. The prevalence of adult residents (more than 20 years old) was obviously higher than young people (less than 20 years old) (Yajuan and Jun, 2004). Therefore, respondents of this study which fall under age range of more than 20 years old were more prone to get affected with the As health related problems if the exposure is increasing.

### 5.1.2 Shrimp paste frequency intake of respondents

This study found that majority of respondents consumed shrimp paste everyday (38%) and 2 to 6 times a week (40%). Totally, about 78% of respondents frequently consume shrimp paste in their daily diet. This frequency intake of shrimp paste in study areas were supported with the finding on study on Perceptions and Acceptance of Shrimp Paste (*belacan*) in Malaysian dishes. The study finding demonstrated that majority of the respondents (34.1%) consumed dishes with shrimp paste 2 to 3 times in a week while 26.7% said that they consumed shrimp paste dishes 2 to 3 times in a month. From the findings, it was found that shrimp paste was relatively favourable to majority of the respondents as more than 40% of the respondents consumed dishes with shrimp paste more than 2-3 times in a week (Leong *et al.*, 2009).

In this study, the frequency intake of shrimp paste of respondents was categorized into “frequent” and “less frequent”. Respondents who consume shrimp paste everyday and 2 to 6 times a week categorized as “frequent” intake and the rest

of respondents who take once a week, 2 to 3 times a month and once a month and seldom categorized as “less frequent” intake. However, majority of respondent (78%) consume shrimp paste everyday and 2 to 6 times a week.

The frequency intake of shrimp paste is important to be known in order to determine the ADD and LADD of each respondent. The information on ADD is needed for the estimation of respondents' non-carcinogenic health risk. USEPA Integrated Risk Information System (IRIS) defined ADD as the dose rate averaged over a pathway-specific period of exposure expressed as a daily dose on a per-unit-body-weight basis and Lifetime Average Daily Dose (LADD) is the estimated dose to an individual averaged over a lifetime of 70 years; used in assessments of carcinogenic risk.

Arsenic exposure might come from other types of food other than shrimp paste. Based on the result on top 10 types of food which frequently consume by respondents, there were several kind of food which have high potential to contaminated with arsenic. It includes rice, vegetables, and marine fish. Several studies showed that there were contaminations of arsenic in rice. Arsenic pollution does not stop at the level of potable water sources. Recent surveys have suggested that arsenic is entering into food sources, in particular, rice. This is mainly due to the use of arsenic-rich groundwater for the irrigation of paddy fields, which effectively is resulting in an increase of arsenic in soils and rice plants (Abedin *et al.*, 2002,

Meharg and Rahman, 2003). Study by Misbahuddin *et al.*, (2003) and Ackerman *et al.*, (2005) found that subsequent cooking of rice in contaminated water may even boost its arsenic level by an additional 10- 35%.

Based on the result of food frequency intake, it revealed that vegetables were one of the frequent kind of food consumes by respondents in this study. There were few studies found that the vegetables also pose arsenic exposure to the community. In general, arsenic content in plants varied considerably with type of plants, type of soil, and arsenic content of irrigation-water. It was believed that vegetables could become contaminated by mining, vehicular exhaust, industrial activities and other agriculture activities (Cui *et al.*, 2004; Li *et al.*, 2006).

Based on Li *et al.*, (2006), Wang *et al.*, (2008) and Xie *et al.*, (2006), studies revealed that the levels of heavy metals in vegetables and soils and their risk to people were of great public concern in China. That finding also supported by Imamul *et al.*, (2006). They found that, the highest concentrations of arsenic were always recorded in plant-roots in Bangladesh, and this may be attributed to contamination from fine colloidal particles. Peeled vegetable samples also showed concentration of arsenic higher than the Australian permissible levels (1 mg/kg fresh weight), indicating significant accumulation in the plant tissues. Furthermore, the findings from the survey of arsenic and other heavy metal in food composites and drinking water in

arsenic-affected area of West Bengal, India showed the mean value of arsenic levels in vegetables was 20.9 mg/kg (Roychowdhury *et al.*, 2002).

Indeed, the study finding exhibit that 72% of respondents consume marine fish in their daily diet intake. By referring to previous study on As contamination, fish was known to be a common sources of As exposure to the community. According to the study by Pavelka and Sedláček (1976) on arsenic contamination in imported sea fish and fish products, the rate of arsenic contamination in sea-fish is relatively high compared with freshwater fish. It also supported by US FDA which stated that in examining the food category in more detail showed that fish and other seafood account for 90% of the total food arsenic exposure with all other foods accounting for the remaining 10% (US FDA, 1993). Besides, in UK, fish is the main contributor of arsenic in the diet. The average concentrations of total and inorganic arsenic in fish were 3214 micrograms/kilogram and 16 mg/kg respectively, whilst total arsenic concentrations in other food groups were considerably lower - the second highest level of 73 mg/kg was found in poultry (FSA, 2005).

All these showed that the As contamination in rice and other type of food had occurred in few countries and indirectly indicated that community had exposed to a variety of As exposures in a their daily food sources.

### 5.1.3 Arsenic concentration in shrimp paste

Arsenic concentration detected using the ICP-MS was demonstrated in ppb. However, the value did not show the actual concentration of As concentration in the shrimp paste sample. It is because; the sample was initially diluted before it was introduced to ICP-MS. Thus, the raw value of As obtained from the ICP-MS must be converted into mg/kg by using following equation A to obtained the actual concentration of As in the shrimp paste sample.

This study showed that mean of As concentration of KP (3.94mg/kg) was slightly higher compared to PR (3.43mg/kg). The shrimp paste from KP was produced by the nearest local factory whereas the shrimp paste from PR was prepared by the villagers themselves. The differences detection of As in the factory-made shrimp paste and home-made shrimp paste might be due to the differences in the raw ingredients used and making process. Based on the interview with the respondents, they claimed that the factory-made shrimp paste was added with some food preservative and did not use the local sources of shrimp as their raw ingredients.

There were studies which showed the highest concentration of As in shrimp compared to other kinds of seafood. Based on study by Attar *et al.*, (1992), As levels in marine organisms are influenced by species differences, size of organism, and human activities. Bottom dwellers such as shrimp, crab, and lobster accumulate more

arsenic than fish due to their frequent contact with bottom sediments Shrimp constitute approximately 30% of mean total seafood consumption in Kuwait (Khordagui and Al-Ajmi, 1991). Also, it is lower than the mean arsenic concentration in shrimp (*Penaeus semisulcatus*), lobster (*Thenus orientalis*) and crab (*Lupa pelagica*) from the Saudi Arabian marine environment (Attar *et al.*, 1992).

Apart from that, according to the Malaysia Food Regulation 1985, the maximum level for Arsenic detected in food was 1 mg/kg. However, arsenic concentrations in all shrimp paste samples exceed the permitted level stated in Malaysia Food Regulation 1985. This result was similar with the finding on toxicological evaluation of some Malaysian locally processed raw food products. The levels of total arsenic in each food item were much higher compared to the level permitted by the Malaysian Food Act 1983 (1 mg/kg). Shrimp pastes contained the highest level of total arsenic with 6.16 mg/kg followed by dried shrimps with 4.03 mg/kg and salted fish which contained the least (1.89 mg/kg) (Sharif *et al.*, 2005).

However, the higher arsenic concentration detected in the sample might be due to the instrument and chemical interference. The facts that ICP-MS interference in the As detection also supported by the Rasmussen *et al.*, (2004) on Environmental Health And Human Exposure Assessment which stated that the ICP-MS is a very sensitive analytical technique for multi-element analysis, but is susceptible to isobaric interferences, either from isotopes of different elements or from polyatomic

ions in the form of molecular or doubly charged ions. For arsenic analysis, hydrochloric acid and perchloric acid should not be used for sample preparation because of the formation of argon chloride from argon in the plasma, which can lead to measurement problems. ArCl has a mass of 75, the same as arsenic, which could lead to errors. Correction for isobaric interferences is possible using either high resolution ICP-MS or correction equations after measurement of the possible interferences. Therefore, whenever possible, nitric acid should be used in sample preparation (Rasmussen *et al.*, 2004).

Overall, As was detected in all shrimp paste samples and exceed the maximum level of As concentration in food stated in Malaysia Food Regulation 1985 (1 mg/kg).

#### **5.1.4 Prevalence of acute and chronic arsenic poisoning signs among respondents.**

Overall, less than 30% of respondents perceived the signs of acute and chronic As poisoning. However, the signs of acute arsenic poisoning were similar with the common signs of other diseases. Hence, we cannot simply diagnosed the respondents who had the symptoms might be caused by the As exposure in their diet. Indeed, there was only questionnaire based and no clinical test to analyze the biological sampling being done in order to confirm the symptoms. According to Mueller, on

toxicology causes of acute abdominal disorders, the clinical features initially invariably relate to the gastrointestinal system and are nausea, vomiting, colicky abdominal pain, and profuse watery diarrhoea. Besides, the abdominal pain may be severe and mimic an acute abdomen. Other clinical features are acute psychosis, a diffuse skin rash, toxic cardiomyopathy, and seizures (Ghariani M, *et al.*, 1991).

The symptoms may be experienced almost immediately after consuming drink or food containing arsenic or it may only be present 30 to 60 minutes after consumption. This depends on the quantity of arsenic within the food or drink and the type of food ingested (Chris, 2011). According to Food and Environmental Hygiene Department of Hong Kong (FEHD), symptoms of acute toxicity include severe inflammation of gastrointestinal tract, leading to severe vomiting and diarrhoea, often with blood-tinged stools. This can be accompanied by secondary electrolyte disturbances with clinical features of muscular cramps, facial oedema and cardiac dysfunction. Sensory loss is one of the neurological presentations of arsenic intoxication. It has been reported that the fatal dose of ingested arsenic trioxide ranges from 70 to 180 mg (FEHD, 2002).

Furthermore, chronic exposure to inorganic arsenic compounds is associated with skin lesions, hyperkeratosis and chronic pathological liver changes. A high prevalence of a peripheral vascular disease called “blackfoot disease” was found in a population living in Taiwan, where the speculated causative factor was related to the

arsenic exposure via drinking well water. A few observations of increased incidence of leukaemia and lung cancers suggested that inorganic arsenicals might be considered as cancer promoter instead of initiators (FEHD, 2002). Simon *et al.*, (2006) stated that, in follow-up visits, people that were exposed to high levels of As from drinking water and/or food for many years were frequently developing cancer. These small communities in West Bengal use groundwater sources for drinking, and this study showed that intervention of water management is critical (Simon *et al.*, 2006). Tsuji, (2005) revealed that the epidemiological data found that arsenic has a little evidence with cancer risk.

According to the article on symptoms, disease and treatment on Healthype.com by Dr. Chris, (2011) usually arsenic poisoning, whether accidental or intentional, occurs over a long period of time. Small doses of arsenic in contaminated drinking water accidentally or intentionally added to daily meals leads to chronic arsenic poisoning. The signs and symptoms of arsenic poisoning in these cases develop over a period of time and are often mistaken for other conditions like gastrointestinal, neurological or immune related. Besides that, there was study which highlights that prevalence of As health related problems were associated with the concentration of As in the drinking water. It stated in the large population based studies from West Bengal in India show a relationship between arsenic concentration in tube well water, dose per body weight, and the prevalence of hyperpigmentation and keratosis (Chris, 2011).

Research by Ratnaike (2003) illustrated that long term arsenic toxicity leads to multisystem disease and the most serious consequence was malignancy. It also stated that the clinical features of arsenic toxicity vary between individuals, population groups, and geographic areas and it was unclear factors that determine the occurrence of a particular clinical manifestation or which body system was targeted. Thus, it was common when persons exposed to chronic arsenic poisoning experienced a wide range of clinical features. That study also demonstrated that the onset is insidious with non-specific symptoms of abdominal pain, diarrhoea, and sore throat. Besides that, Lien *et al.*, (1999) found that numerous skin changes occur with long term exposure to As. Dermatological changes also reported by Guha *et al.*, (1998). That study found that it was common feature and the initial clinical diagnosis was often based on hyperpigmentation palmar and solar keratosis.

According to Smith *et al.*, (2000), hyperpigmentation occurs as diffuse dark brown spots, or less discrete diffuse darkening of the skin, or has a characteristic “rain drop” appearance. Besides that, Abernathy *et al.*, (1999) found that As associated skin cancer, Bowen’s disease, is an uncommon manifestation in Asians and may be due to the high skin melanin content and increased exposure to ultraviolet radiation. It was reported that As may cause a basal cell carcinoma in a non-melanin pigmented skin.

Overall, it can be concluded that there were signs of As health related problems detected among the respondents. However, all the reported health problems cannot be confirmed as As poisoning since there was no clinical test done to the respondents.

### 5.1.5 Health risk of respondents

In order to determine the health risk of respondents toward the carcinogenic and non-carcinogenic health effect, firstly, there must be the calculation of Lifetime Average Daily Dose (LADD) and Average Daily Dose (ADD) respectively by using equation I and II. Next, the value would be inserted into the equation III for Hazard Quotient (HQ) and equation IV for Lifetime Cancer Risk (LCR) respectively. Finally, for the carcinogenic health risk, the value obtained from equation IV would be compared with 3 level of risk stated in the 3 classification of LCR which were clearly acceptable, acceptable and clearly unacceptable. For the non-carcinogenic health effects, the value obtained from equation III would be compared with 2 classification of HQ which either acceptable or unacceptable risk.

Based on ADD and LADD value of respondents, the mean were  $1.4 \times 10^{-6}$  mg/kg and  $7 \times 10^{-7}$  mg/kg respectively which not exceeded Provisional Tolerable Weekly Intake (PTWI) for As which is 0.015mg/kg. PTWI was defined as the acceptable level of toxic metal that can be ingested on a weekly basis, as determined

by the World Health Organization and the Food and Agriculture Organization. It expressed as a weekly basis to emphasize that long term exposure is important for contaminants that cumulate in the day. It provides a bright line for the risk manager against which intake can be compared.

This study found that all of respondents fall under clearly acceptable and acceptable risk towards the carcinogenic health effects (LCR). It might be due to the low level of arsenic concentration detected in shrimp paste sample in study areas and the lifetime average daily dose (LADD) of the respondents and unlikely to cause adverse health effects to respondents. Furthermore, the shrimp paste only taken in a very small amount to be added in the daily meals. Hence, the carcinogenic risk faced by the respondents considered as acceptable risk. On the other hand, Obiri *et al.*, (2005) who studied among Obuasi Municipality's community, Ghana found that the carcinogenic health risk of As exposure exceed the USEPA's acceptable cancer risk range of  $1 \times 10^{-6}$  to  $1 \times 10^{-4}$ .

For non carcinogenic health risk (HQ), result also depicted that all the respondents were under acceptable risk. It shows that the respondents unlikely to get affected by non-carcinogenic health effects. However, the study by Obiri *et al.*, (2005) demonstrated that the the results of the non-cancer human health risk towards As exposure through water sources were also found in most cases to be greater than the USEPA's acceptable non-cancer human health hazard index of 1.

According to the toxicological profile of As by ATSDR (2007), the most characteristic effect of long-term oral exposure to inorganic arsenic compounds is the development of skin lesions; these lesions are often used as diagnostic criteria for arsenicosis. These include patches of darkened skin and are often associated with changes in the blood vessels of the skin and caused the developing of skin cancer. Besides, swallowing arsenic has also been reported to increase the risk of cancer in the liver, bladder, and lung.

Although all respondents still under acceptable risk to get affected with carcinogenic and non-carcinogenic health risk at the current moment, it did not guarantee that the risk will remain at acceptable level in the future since the As contaminated-shrimp paste was continuously consumed by the community.

#### **5.1.6 Relationship between the shrimp paste frequency intake and the health risk encountered by respondents.**

This study found that there was no significant relationship between the shrimp paste frequency intake and the lifetime cancer risk (LCR) for carcinogenic health risk. Similarly, there was no significant relationship found between the shrimp paste frequency intake and the hazard quotient (HQ) for non-carcinogenic health risk. It indicated that carcinogenic (LCR) and non-carcinogenic health risk (HQ) encountered by the respondents did not fully depend on the shrimp paste frequency

intake in their daily diet. It was because the acute symptoms which perceived by respondents might be caused by other sources and other routes of As exposure. On the other hand, the health risk of respondents only accounted for consumption of As contaminated-shrimp paste without take into account other As exposure sources such as drinking water, and other As contamination food sources.

Furthermore, according to the Cuzik *et al.*, (1992), the exposed population reported high cases of skin cancer compared to unexposed population which illustrated by few epidemiological studies which were conducted in several countries including Taiwan, Mexico, Chile, Hungary, England, Japan, and Argentina. Based on that finding, it exhibits that the health risk regardless of carcinogenic or non carcinogenic effects would increase with the arising of exposure. Hence, the health risk of respondents might elevate if the consideration of other sources of As exposure.

On the other hand, there were several studies which clearly found that the association of As exposure towards an increase of cancer cases among study population. It was supported with the finding by Smith *et al.*, (1992) as the result showed the association of inorganic arsenic ingestion with increasing of mortality cases from internal cancers. By referring to the research plan for As in drinking water by USEPA (1998), it was revealed that an increased prevalence of skin cancer was observed among approximately 40,000 Taiwanese consuming arsenic contaminated

water (up to 1,200 mg/ L arsenic) from artesian wells as compared with approximately 7,500 residents from Taiwan and a neighboring island, Matsu, consuming “arsenic free” (0-17 mg/L arsenic). Based on the finding of all these research, it displays that there is a possibility of association between frequency intakes of As contaminated food with health risk of community.

This study found that health risk did not show any significant relationship with the shrimp paste frequency intake. It was because the dose taken by respondent still not exceeds PTWI level as shown in the table 4.2. At this current moment, the dose of As intake might not cause any health risk to the community. However, the health risk might increase if As exposure occurred continuously and accounted for another route of exposure.

#### **5.1.7 Relationship between shrimp paste frequency intake and the prevalence of acute and chronic arsenic poisoning signs of the respondents.**

This study exhibited that there was no significant relationship between the shrimp paste frequency intake and the prevalence of possible acute and chronic arsenic poisoning signs found except for the diarrhea signs. However, there were few studies depicted that the high exposure to Arsenic caused the increasing of As poisoning symptoms among respondents.

According to WHO, intake of 1.0 mg of inorganic arsenic per day may give rise to skin lesions within a few years. The Food and Agriculture Organization/World Health Organization (FAO/WHO) provisional tolerable weekly intake (PTWI) of arsenic translated to a daily basis, is 2.1 mg/kg body wt./day (Joint FAO/WHO, 1989, Roychowdhury T. *et al.*, 2002). A large number of epidemiological investigations have confirmed that high concentration of As are associated with peripheral vascular diseases (Xia *et al.*, 1995), polyneuropathy (Li and Wang, 1993), hypertension and a high risk of skin cancers and other cancers (Luo *et al.*, 1995; Chen *et al.*, 1996; Chiou and Xue, 1996).

Furthermore, based on study on clinical observations and medical outcomes in 149 cases of arsenate ant killer ingestion, most cases of acute arsenic poisoning occur from accidental ingestion of insecticides or pesticides, Small amounts (<5 mg) result in vomiting and diarrhoea but resolve in 12 hours and treatment is reported not to be necessary (Kingston *et al.*, 1993). Besides that, there were studies by international health bodies which illustrated that As can give carcinogenic effects to the community. For instance, the Department of Health and Human Services (DHHS) has determined that inorganic arsenic is known to be a human carcinogen (Eco USA, 1996). Inorganic arsenic is more toxic than organic forms and inorganic arsenic is classified by the International Agency for Research on Cancer (IARC) as "carcinogenic to humans" (Group 1) on the basis of "sufficient evidence" for an increased risk for cancer of the urinary bladder, lung and skin (IARC, 2002). Besides

cancer induction, chronic human exposure to arsenic in drinking water mainly inorganic arsenic has also been associated with peripheral vascular diseases, cardiovascular diseases and possibly with diabetes and reproductive effects (IARC, 2002).

EPA also has classified inorganic arsenic as a known human carcinogen. Almost no information is available on the effects of organic arsenic compounds in humans. Studies in animals show that most simple organic arsenic compounds (such as methyl and dimethyl compounds) are less toxic than the inorganic forms. In animals, ingestion of methyl compounds can result in diarrhea, and lifetime exposure can damage the kidneys. Lifetime exposure to dimethyl compounds can damage the urinary bladder and the kidneys (Eco USA, 1996). Arsenic and its metabolite are chemicals that bioaccumulate in tissues of aquatic organisms but do not biomagnify in the aquatic food chain. The average biological half-life is about 60 days (rats/rabbits) due to the accumulation of arsenic in the erythrocytes. For humans, half-life is shorter because of a fast excretion of arsenic (GTZ, 2010).

Overall, this study did not show significant association between shrimp paste frequency intake with the prevalence of acute and chronic arsenic poisoning signs of the respondents. However, the association might be significant if the exposure time is longer as the As accumulation increasing per day.

## 5.2 Conclusion

All shrimp paste samples contained As which exceed the permitted level in food stated in Malaysia Food Regulation 1985. Based on the HQ and LCR, all respondent falls under acceptable risk which indicates that the frequency intake of shrimp paste unlikely to cause adverse health effects to the respondents. There was no significant relationship observed between the shrimp paste frequency intake and the health risk encountered by respondents. However, the biological sampling and clinical test could be done for future studies in order to confirm As body burden of respondents and associated it with the shrimp paste frequency intake.

To encapsulate, all respondents are considered to be safe from the risk of As related health problems at this current moment. This is because, only small amount of shrimp paste was consumed which later might accumulated in the body which can cause adverse health effect to the body. Indeed, the mean of LADD and ADD for the consumption of shrimp paste were  $8 \times 10^{-7} \pm 4 \times 10^{-7}$  and  $1.4 \times 10^{-6} \pm 5 \times 10^{-7}$  respectively which not exceeded PTWI for As which is 0.015mg/kg. The monitoring on the contamination of As in local processed food generally or shrimp paste sample specifically by the related authorities is very significant in order to ensure that As level is below the permitted level in order to ensure that there is no adverse health risk to the community.

### 5.3 Recommendations

There are several improvements should be done in term of methodology, and study design in order to improve this study in the future for better findings. In this study, although high As concentration detected in the shrimp paste sample, we cannot trace the actual As sources as it might be from the shrimp as the raw ingredients, water sources, or from the other environmental elements. Hence, in terms of study design, the future study should highlight the possible sources of As which caused the high As detection in shrimp paste. For example, the study is about to determine the As concentration contained in shrimp and water sources where high As concentration detected in sample of the study area. With that, we can conclude what is actually the sources of As which contributed to the high As concentration in shrimp paste.

First and foremost, to strengthen the methodology of this study in demonstrating the actual health risk encountered by community, the biological monitoring should be conducted to determine the actual body burden on the As exposure. For instance the blood or nail sample can be analysed to detect the As level in the body. By doing that, we can determine the As accumulation in the respondents who frequently consumed As contaminated shrimp paste. Hence, we can associate the acute and chronic As poisoning symptoms that perceived by respondents with the As level accumulated in the body.

Nowadays, in the era of globalization and technological advancement, it was impossible to totally eliminate exposure of community towards heavy metals either through environmental or food intake. Hence, the community themselves should take own initiatives in preventing the overexposure to heavy metals particularly in nutritional aspects. There are few nutritional intakes that are proved to detoxify the arsenic accumulation in the body. Firstly, the community are advocated to consume sulphur rich foods such as eggs, garlic, onions, poultry and eggs, fish, beans and legumes Sulphur protects cells from the effects of toxins and it assists in the formation of bile. Next, the community also should increase their fiber rich foods intake. Fiber aids in detoxification since toxins will adhere to fiber and be eliminated as waste (Katelyn, 2012). The consumption of vegetables and fruits provide ample sources of fiber. Furthermore, the intake of nutrients likes selenium, iodine, calcium, zinc, and vitamin C can acts as protective agents against the effects of arsenic (Jeremy, 2011).

Besides, regular enforcement by authorities concerned such as Ministry of Health and Health Unit of city or district Municipal should be conducted particularly in the favourable local processed food. It is to ensure that the nutritional contents do not violate the maximum level as stated in Food Regulation 1985. On the other hand, the manufacturer themselves should take precautionary measures and action especially in hygiene aspects during preparation of their products and also in choosing raw ingredients for their products.

Lastly, listed recommendations are very important to be implemented in the future study for the purpose of ensuring that community are not exposed to the high level of As contained either in the food products or in the environmental elements.



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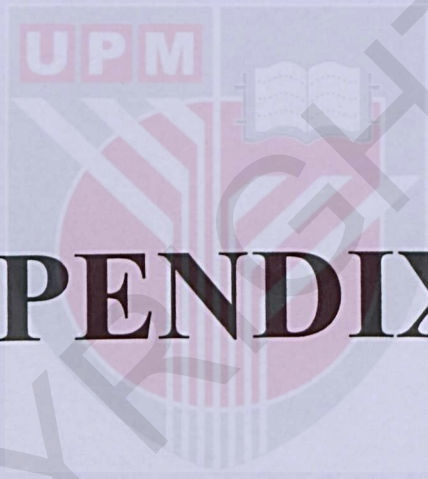
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# APPENDIX I



UPM

## PENERANGAN KEPADA PESERTA

**TAJUK KAJIAN: KEPEKATAN LOGAM BERAT ARSENIK DI DALAM BELACAN DAN  
PENILAIAN RISIKO KESIHATAN DI KALANGAN PENDUDUK DI  
MELAKA.**

**PENYELIDIK : AZIEMAH BT ZULKIFLI**

Terima kasih kerana membantu kami di dalam kajian ini.

### **Apakah kajian ini?**

Sejak kebelakangan ini, beberapa kajian telah membuktikan bahawa terdapat pencemaran logam berat seperti arsenik didalam sumber makanan harian. Ini disebabkan oleh berlakunya pencemaran terhadap sumber-sumber semulajadi seperti sumber air. Secara tidak langsung, keadaan ini telah menyumbang kepada pencemaran logam berat kepada hidupan laut seperti udang geragau yang menjadi bahan asas kepada pembuatan belacan. Pendedahan kepada logam berat arsenik mungkin akan berlaku kepada masyarakat yang menjadikan belacan sebagai salah satu menu dalam pemakanan seharian mereka. Sehubungan dengan itu, kajian ini dijalankan adalah bertujuan untuk menentukan kepekatan logam berat arsenik yang terkandung di dalam belacan dan menjalankan penilaian risiko kesihatan terhadap pengambilan belacan di dalam menu seharian.

### **Apakah tujuan kajian ini?**

Kajian ini dijalankan bertujuan untuk menentukan kepekatan logam berat arsenik yang terkandung di dalam belacan dan menjalankan penilaian risiko kesihatan terhadap penduduk Melaka.

### **Berapa ramai responden yang terpilih?**

Responden akan dipilih daripada kalangan penduduk yang tinggal berdekatan dengan kawasan pemprosesan belacan di sekitar Melaka iaitu di Kg. Pantai Dalam dan Kg. Pinang. Seramai 101 orang responden dari Kg. Pinang dan seramai 105 orang dari Kg. Pinang akan dipilih untuk kajian ini.



### **Apakah jenis ujian yang akan dijalankan?**

Satu set borang soal kaji selidik akan diberikan kepada setiap responden untuk diisi. Selain daripada itu, pengukuran berat badan, ketinggian, dan tekanan darah akan diambil untuk menetahui tahap kesihatan penduduk dan akan digunakan untuk menganggar risiko kesihatan yang dihadapi oleh penduduk.

### **Adakah bayaran dikenakan?**

Pengkaji akan menanggung segala pembiayaan ujian yang akan dijalankan dan tiada sebarang bayaran dikenakan terhadap setiap responden.

### **Adakah maklumat dijamin sulit?**

Semua maklumat yang diberikan oleh responden di dalam borang kaji selidik adalah dijamin sulit. Tiada huraian individu akan dibuat pada mana-mana bahagian di dalam kajian atau penerbitan.

### **Adakah hak anda?**

Kajian ini melibatkan anda secara sukarela. Oleh itu, peserta mempunyai hak untuk menarik diri dari penyertaan dalam kajian ini pada bila-bila masa sekiranya peserta merasa tidak selesa untuk memberikan maklumat kepada pengkaji.

### **Apakah yang harus anda lakukan?**

Anda dikehendaki menandatangani borang penyertaan responden yang menyatakan minat anda untuk menyertai kajian ini. Ianya boleh dilakukan setelah anda membaca dan memahami isi kandungan penerangan ini. Borang penyertaan responden haruslah dikembalikan kepada penyelidik sebelum ujian dijalankan. Sekiranya anda mempunyai sebarang kemusykilan, penyelidik akan membantu untuk memberi maklumat yang selanjutnya.



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

Terima kasih atas kerjasama dan bantuan anda.

**AZIEMAH BINTI ZULKIFLI**

Penyelidik

B. Sc. Kesihatan Persekitaran dan Pekerjaan

Unit Kesihatan Persekitaran dan Pekerjaan

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saliza@medic.upm.edu.my



**BORANG PERSETUJUAN RESPONDEN**

**TAJUK KAJIAN: KEPEKATAN LOGAM BERAT ARSENIK DI DALAM BELACAN DAN  
PENILAIAN RISIKO KESIHATAN DI KALANGAN PENDUDUK DI  
MELAKA.**

**PENYELIDIK : AZIEMAH BT ZULKIFLI**

Saya ..... No.K/P: .....  
alamat.....

.....dengan ini secara sukarela bersetuju untuk mengambil bahagian dalam penyelidikan kajian soal selidik yang dinyatakan di atas. Saya telah dimaklumkan mengenai latar belakang penyelidikan ini dari segi kaedah, kemungkinan kesan buruk dan komplikasi( rujuk kepada risalah maklumat). Saya faham bahawa saya mempunyai hak untuk menarik diri dari kajian ini pada bila-bila masa tanpa memberikan apa jua sebab. Saya juga faham bahawa kajian ini adalah sulit dan semua maklumat yang diberikan mengenai identiti saya adalah sulit dan persendirian.

Saya ingin \*tahu/tidak ingin mengetahui keputusan ujian yang dijalankan ke atas sampel saya.

\* potong mana yang tidak berkaitany

Tandatangan .....  
(Responden)

Tandatangan.....  
(Saksi)

Tarikh : .....

Nama : .....

No. K/P : .....

Saya mengesahkan bahawa saya telah menjelaskan kepada responden latar belakang dan tujuan penyelidikan di atas.

Tarikh .....

Tandatangan.....  
(Penyelidik)



**BORANG PERSETUJUAN IBUBAPA/GRADUAN**

**TAJUK KAJIAN: KEPEKATAN LOGAM BERAT ARSENIK DI DALAM BELACAN DAN  
PENILAIAN RISIKO KESIHATAN DI KALANGAN PENDUDUK DI  
MELAKA.**

**PENYELIDIK : AZIEMAH BINTI ZULKIFLI**

Saya ..... No.K/P: .....  
alamat.....

.....dengan ini secara sukarela bersetuju untuk mengambil bahagian dalam penyelidikan kajian soal selidik yang dinyatakan di atas. Saya telah dimaklumkan mengenai latar belakang penyelidikan ini dari segi kaedah, kemungkinan kesan buruk dan komplikasi( rujuk kepada risalah maklumat). Saya faham bahawa saya mempunyai hak untuk menarik diri dari kajian ini pada bila-bila masa tanpa memberikan apa jua sebab. Saya juga faham bahawa kajian ini adalah sulit dan semua maklumat yang diberikan mengenai identiti saya adalah sulit dan persendirian.

Saya ingin \*tahu/tidak ingin mengetahui keputusan ujian yang dijalankan ke atas sampel saya.

\* potong mana yang tidak berkaitany

Tandatangan .....  
(Ibubapa/Graduan)

Tandatangan.....  
(Saksi)

Tarikh :.....

Nama :.....

No. K/P :.....

Saya mengesahkan bahawa saya telah menjelaskan kepada responden latar belakang dan tujuan penyelidikan di atas.

Tarikh .....

Tandatangan.....  
(Penyelidik)



ID Responden:

**JABATAN KESIHATAN KOMUNITI  
FAKULTI PERUBATAN DAN SAINS KESIHATAN  
UNIVERSITI PUTRA MALAYSIA**

**Kajian kepekatan logam berat arsenik dan risiko kesihatan kepada penduduk di Melaka.**

Adalah dimaklumkan bahawa satu kajian tentang kesihatan akibat pendedahan kepada kepekatan arsenik dalam belacan sedang dijalankan di tempat anda. Sehubungan dengan itu, sukacita dimaklumkan bahawa anda telah terpilih untuk menjadi salah seorang responden kajian ini. Oleh demikian, anda diminta menjawab semua soalan yang dikemukakan dengan mengikut arahan yang telah diberikan. Segala maklumat berkenaan responden akan dirahsiakan dan hanya akan digunakan untuk kajian ini.

ID Responden:

Tarikh kajian: .....

No Kad Pengenalan:

No Telefon: (R)

(Hp)

BAHAGIAN A	KETERANGAN DIRI	RUANGAN KOD																				
<p>Tandakan (√) dalam kotak berkenaan.</p> <p>Alamat Semasa: .....</p> <p>.....</p> <p>1. Umur: ..... Tahun</p> <p>2. Jantina: <input type="checkbox"/> Lelaki                      <input type="checkbox"/> Perempuan</p> <p>3. Bangsa: <input type="checkbox"/> Melayu                      <input type="checkbox"/> Cina                      <input type="checkbox"/> India</p> <p>                  <input type="checkbox"/> Lain-lain (Sila nyatakan): .....</p> <p>4. Agama:</p> <p><input type="checkbox"/> Islam</p> <p><input type="checkbox"/> Kristian</p> <p><input type="checkbox"/> Buddha</p> <p><input type="checkbox"/> Hindu</p> <p><input type="checkbox"/> Lain-lain</p> <p>5. Status:</p> <p><input type="checkbox"/> Bujang</p> <p><input type="checkbox"/> Berkahwin</p> <p><input type="checkbox"/> Bercerai/berpisah</p> <p><input type="checkbox"/> Balu/Duda</p> <p>6. Taraf pendidikan:</p> <table data-bbox="224 1422 1050 1627"> <tr> <td><input type="checkbox"/></td> <td>Tidak Bersekolah</td> <td><input type="checkbox"/></td> <td>Diploma</td> </tr> <tr> <td><input type="checkbox"/></td> <td>UPSR</td> <td><input type="checkbox"/></td> <td>Ijazah Sarjana Muda</td> </tr> <tr> <td><input type="checkbox"/></td> <td>PMR</td> <td><input type="checkbox"/></td> <td>Sarjana Muda</td> </tr> <tr> <td><input type="checkbox"/></td> <td>SPM</td> <td><input type="checkbox"/></td> <td>Doktor Falsafah</td> </tr> <tr> <td><input type="checkbox"/></td> <td>Sijil/STPM/Matrikulasi</td> <td></td> <td></td> </tr> </table> <p>7. Jenis pekerjaan:</p> <p><input type="checkbox"/> Kerajaan</p> <p><input type="checkbox"/> Swasta</p> <p><input type="checkbox"/> Bekerja Sendiri</p> <p><input type="checkbox"/> Pencen/Tidak Bekerja</p> <p><input type="checkbox"/> Lain-lain</p> <p>8. Pendapatan sebulan (RM) : _____</p>		<input type="checkbox"/>	Tidak Bersekolah	<input type="checkbox"/>	Diploma	<input type="checkbox"/>	UPSR	<input type="checkbox"/>	Ijazah Sarjana Muda	<input type="checkbox"/>	PMR	<input type="checkbox"/>	Sarjana Muda	<input type="checkbox"/>	SPM	<input type="checkbox"/>	Doktor Falsafah	<input type="checkbox"/>	Sijil/STPM/Matrikulasi			
<input type="checkbox"/>	Tidak Bersekolah	<input type="checkbox"/>	Diploma																			
<input type="checkbox"/>	UPSR	<input type="checkbox"/>	Ijazah Sarjana Muda																			
<input type="checkbox"/>	PMR	<input type="checkbox"/>	Sarjana Muda																			
<input type="checkbox"/>	SPM	<input type="checkbox"/>	Doktor Falsafah																			
<input type="checkbox"/>	Sijil/STPM/Matrikulasi																					

9. Pendapatan isi rumah sebulan (RM) : _____	
10. Bilangan ahli isirumah : _____ orang	

BAHAGIAN B	MAKLUMAT KESIHATAN	RUANGAN KOD
------------	--------------------	-------------

1. Adakah anda mengalami masalah kesihatan berikut?

Simptom akut:

- Sakit perut
- Muntah
- Cirit-birit
- Kekejangan otot
- Pening
- Kesukaran bernafas
- Kelesuan
- Alergi
- Rambut mudah gugur

Simptom kronik:

- Kulit di tapak tangan & kaki menjadi tebal (hyperkeratosis)
- Bintik-bintik di permukaan kulit
- Kuku rapuh
- Kebas di bahagian tangan
- Kanser, nyatakan : \_\_\_\_\_
  
- Hilang fokus
- Mudah lupa
- Kebas dan semut-semut dikaki
- Kabur penglihatan
- Pucat
- Bengkak pada bahagian kaki dan muka

2. Adakah anda telah mendapatkan rawatan untuk symptom-symptom diatas?

- Ya, nyatakan kali terakhir anda mendapatkan rawatan \_\_\_\_\_
- Tidak

BAHAGIAN C:	ANTHROPOMETRI	RUANGAN KOD
1. Berat (kg) :	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> kg	
2. Tinggi (cm) :	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> cm	

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

$$\text{BMI} = \frac{\text{Berat (kg)}}{\text{Tinggi} \times \text{Tinggi}(\text{m}^2)}$$

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

**BAHAGIAN D: BORANG KEKERAPAN PENGAMBILAN MAKANAN**

Kod	Jenis makanan	Kekerapan pengambilan					Ukuran sajian(pilih satu jenis ukuran sahaja)	Berapa banyak sajian setiap kali makan
		Berapa kali sehari	Berapa kali seminggu	Berapa kali sebulan	Berapa kali setahun	Tidak makan		
A1	Nasi						Pinggan	
							Mangkuk cina	
							Cawan	
							senduk	
A2	Bijirin						Pinggan	
							Mangkuk cina	
							Cawan	
							Senduk	

Kod	Jenis makanan	Kekerapan pengambilan					Ukuran sajian(pilih satu jenis ukuran sahaja)	Berapa banyak sajian setiap kali makan
		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	Ikan dalam tin						Ekor	
B5	Kekerang						Sudu makan	
B6	Udang basah						Ekor sederhana	
B7	Sotong basah						Potong sederhana	
B8	Sotong kering						Keping sederhana	
							Potong sederhana	
B9	Ketam						Ekor	
B10	Ikan kering						Keping	
							Ekor	
B11	Bebola ikan/kek ikan						Bebola	
							Ketul	
B12	Kerepok lekor						Ketul	

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

Kod	Jenis makanan (C) Sayuran	Kekerapan pengambilan					Ukuran sajian (pilih satu jenis ukuran sahaja)	Berapa banyak sajian setiap kali makan
		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 (E)Makanan perencah /perasa	Kekerapan pengambilan					Ukuran sajian(pilih satu jenis ukuran sahaja)	Berapa banyak sajian setiap kali makan
		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 teh	
E4	Kicap pekat						Sudu teh	
E5	Kicap cair						Sudu makan	
E6	Sos cili/tomato						Sudu makan	
E7	Sos tiram						Sudu teh	

Kod	Jenis makanan (D)Makanan yang dikaji	Kekerapan pengambilan					Ukuran sajian(pilih satu jenis ukuran sahaja)	Berapa banyak sajian setiap kali makan
		Berapa kali sehari	Berapa kali seminggu	Berapa kali sebulan	Berapa kali setahun	Tidak makan		
F1	Budu							

Sumber bekalan budu:

Buatan sendiri

Perusahaan kecil (IKS)

Nyatakan 3 jenis jenama budu yang anda guna:

1. \_\_\_\_\_
2. \_\_\_\_\_
3. \_\_\_\_\_

Sudah berapa lamakah anda mengambil budu sebagai salah satu menu harian?

Sila nyatakan : \_\_\_\_\_

## LAMPIRAN 1

Berikut adalah butir-butir pelajar dan Projek Ilmiah Tahun Akhir yang akan dijalankan:

1. Nama Pelajar : **Aziemah Binti Zulkifli**
2. No. Matrik/ Kad Pengenalan : 147318/ 891018-03-5466
3. No. Tel./ Emel : 019-9312144/ azie\_zul5466@yahoo.com
4. Nama Penyelia Projek : Dr. Saliza Mohd Elias
5. No. Tel. Penyelia / Emel : 03-89472402/ saliza@medic.upm.edu.my
6. Tajuk Kajian : **Kandungan Arsenik Di Dalam Belacan Dan Penilaian Risiko Kesihatan Di Dua Buah Kampung Di Melaka**
7. Tempoh Penyelidikan : 15/12/2011 hingga 15/02/2012
8. Bilangan Responden Yang Diperlukan : 127 orang
9. Bahan/ Alat Kajian : Borang soal selidik, Pengukuran berat badan dan ketinggian (BMI)



**GERAKAN BELIA 4B (MALAYSIA)**

Cawangan Pinang Bahagia

Kawasan Tangga Batu

Melaka



Tel. Bimbit : 6012-3653964

E-Mail : alil79\_mrbk@yahoo.com

Bil. Surat Kami :

Bil. Surat Tuan :

Mohd Rasul Bin Hj. Khamis  
Ketua Gerakan Belia 4B ( Malaysia )  
Cawangan Pinang Bahagia  
Kawasan Tangga Batu  
Melaka

17 hb Januari 2012

Cik Aziemah Bt. Zulkifli ( 147318 ) & Cik Nor Aisyah Bt. Abd. Razak (148748 )  
Unit Kesihatan Persekitaran & Pekerjaan,  
Jabatan Kesihatan Komuniti,  
Fakulti Perubatan & Sains Kesihatan,  
Universiti Putra Malaysia,  
43400 Serdang Selangor.

TUAN

**MEMBERIKAN KEBENARAN UNTUK TUAN MEMBUAT KAJIAN DI KAMPUNG PINANG  
TENTANG KEPEKATAN LOGAM BERAT ARSENIK & PLUMBUM DI DALAM BELACAN DAN  
PENILAIAN RISIKO KESIHATAN DI KALANGAN PENDUDUK DI MELAKA.**

Adalah dengan hormatnya, perkara di atas dirujuk.

2. Sehubungan dengan itu, sukacita dimaklumkan bahawa satu kajian di atas oleh tuan akan dijalankan pada masa seperti berikut:

Tarikh : 20 hb hingga 22 hb Januari 2012 ( Jumaat- Ahad )  
Masa : 9.00 Pagi hingga 5.00 Petang  
Tempat : Kampung Pinang

3. Kami dengan sukacitanya memberikan kebenaran kepada tuan untuk menjalankan kajian tersebut di kawasan Kampung Pinang ini. Kami warga belia di sini, juga amat terharu dan berbangga serta ingin mengucapkan jutaan terima kasih di atas kesudian tuan hadir dan memilih membuat kajian di kampung ini. Di harap segala kajian tuan dapat dijalankan dengan baik dan berjaya. Segala kekurangan di sini, kami memohon maaf dan diucapkan jutaan terima kasih di atas kehadiran tuan.

Sekian, terima kasih.

d/a Villa Sri Pinang, Kampung Pinang B, Batu 5 ½,  
76400 Tanjong Keling, Melaka

## 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.	RSD
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		7137.074		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		3630.390		1.8	
Ba++	69.0	4820.6		0.025		0.001		2.7	
Bkgd	220.0	7.9		7.900		0.693		8.8	
Bkgd	8.5	17.4		17.433		2.016		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 [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

### Current Autolens Data

Analyte	Mass	Num of Pts	DAC 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: Blank

Sample Date/Time: Wednesday, March 28, 2012 12:04:15

Sample Description:

Solution Type: Blank

Blank File: C:\Elandata\_LC-ICPMS\DataSet\Nor Aisyah (FPSK)\Blank.006

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)\Blank.006

Tuning File: C:\Elandata\_LC-ICPMS\Tuning\default.tun

Optimization File: C:\Elandata\_LC-ICPMS\Optimize\default.dac

Calibration File:

Calibration Type: External Calibration

## Summary

### Intensities

Analyte	Mass	Meas. Intens.	Mean	Meas. Intens.	RSD	Blank Intensity	Blank Intens. RSD
As	75		216		7.911		
Cd	111		113		14.264		
Pb	208		200		10.149		

### Concentration Results

Analyte	Mass	Net Intens.	Mean	Conc. Mean	Conc. SD	Conc. RSD	Sample Unit
As	75			0			ppb
Cd	111			0			ppb
Pb	208			0			ppb

## Quantitative Analysis - Summary Report

### Sample ID: Std 1 (10 ppb)

Sample Date/Time: Wednesday, March 28, 2012 12:05:58

Sample Description:

Solution Type: Standard

Blank File: C:\Elandata\_LC-ICPMS\DataSet\Nor Aisyah (FPSK)\Blank.006

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)\Std 1 (10 ppb).007

Tuning File: C:\Elandata\_LC-ICPMS\Tuning\default.tun

Optimization File: C:\Elandata\_LC-ICPMS\Optimize\default.dac

Calibration File:

Calibration Type: External Calibration

### Summary

#### Intensities

Analyte	Mass	Meas. Intens.	Mean	Meas. Intens.	RSD	Blank Intensity	Blank Intens.	RSD
As	75		12348		3.975	216.003		7.911
Cd	111		16767		4.184	113.334		14.264
Pb	208		168746		5.967	200.002		10.149

#### Concentration Results

Analyte	Mass	Net Intens.	Mean	Conc. Mean	Conc. SD	Conc. RSD	Sample Unit
As	75		12132.387	10.000	0.40	4.0	ppb
Cd	111		16653.465	10.000	0.42	4.2	ppb
Pb	208		168546.015	10.000	0.60	6.0	ppb

## Quantitative Analysis - Summary Report

### Sample ID: Std 2 (30 ppb)

Sample Date/Time: Wednesday, March 28, 2012 12:07:41

Sample Description:

Solution Type: Standard

Blank File: C:\Elandata\_LC-ICPMS\DataSet\Nor Aisyah (FPSK)\Blank.006

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)\Std 2 (30 ppb).008

Tuning File: C:\Elandata\_LC-ICPMS\Tuning\default.tun

Optimization File: C:\Elandata\_LC-ICPMS\Optimize\default.dac

Calibration File:

Calibration Type: External Calibration

### Summary

#### Intensities

Analyte	Mass	Meas. Intens.	Mean	Meas. Intens.	RSD	Blank Intensity	Blank Intens.	RSD
As	75		37071		2.159	216.003		7.911
Cd	111		51849		4.230	113.334		14.264
Pb	208		525358		1.457	200.002		10.149

#### Concentration Results

Analyte	Mass	Net Intens.	Mean	Conc. Mean	Conc. SD	Conc. RSD	Sample Unit
As	75		36855.453	30.037	0.65	2.2	ppb
Cd	111		51735.611	30.103	1.28	4.2	ppb
Pb	208		525157.706	30.112	0.44	1.5	ppb

## Quantitative Analysis - Summary Report

### Sample ID: Std 3 (50 ppb)

Sample Date/Time: Wednesday, March 28, 2012 12:09:25

Sample Description:

Solution Type: Standard

Blank File: C:\Elandata\_LC-ICPMS\DataSet\Nor Aisyah (FPSK)\Blank.006

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)\Std 3 (50 ppb).009

Tuning File: C:\Elandata\_LC-ICPMS\Tuning\default.tun

Optimization File: C:\Elandata\_LC-ICPMS\Optimize\default.dac

Calibration File:

Calibration Type: External Calibration

### Summary

#### Intensities

Analyte	Mass	Meas. Intens.	Mean	Meas. Intens.	RSD	Blank Intensity	Blank Intens.	RSD
As	75		63909		0.885	216.003		7.911
Cd	111		86427		1.847	113.334		14.264
Pb	208		866919		6.218	200.002		10.149

#### Concentration Results

Analyte	Mass	Net Intens.	Mean	Conc. Mean	Conc. SD	Conc. RSD	Sample Unit
As	75		63692.525	50.531	0.45	0.9	ppb
Cd	111		86313.644	50.064	0.93	1.8	ppb
Pb	208		866718.563	49.913	3.10	6.2	ppb

## Quantitative Analysis - Summary Report

### Sample ID: Std 4 (100 ppb)

Sample Date/Time: Wednesday, March 28, 2012 12:11:09

Sample Description:

Solution Type: Standard

Blank File: C:\Elandata\_LC-ICPMS\DataSet\Nor Aisyah (FPSK)\Blank.006

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)\Std 4 (100 ppb).010

Tuning File: C:\Elandata\_LC-ICPMS\Tuning\default.tun

Optimization File: C:\Elandata\_LC-ICPMS\Optimize\default.dac

Calibration File:

Calibration Type: External Calibration

### Summary

#### Intensities

Analyte	Mass	Meas. Intens. Mean	Meas. Intens. RSD	Blank Intensity	Blank Intens. RSD
As	75	132397	3.491	216.003	7.911
Cd	111	174685	1.445	113.334	14.264
Pb	208	1665278	3.038	200.002	10.149

#### Concentration Results

Analyte	Mass	Net Intens. Mean	Conc. Mean	Conc. SD	Conc. RSD	Sample Unit
As	75	132180.547	101.218	3.54	3.5	ppb
Cd	111	174571.910	100.322	1.45	1.4	ppb
Pb	208	1665078.028	98.901	3.00	3.0	ppb

# Calibration Report

Analyte	Mass	Curve Type	Slope	Intercept	Corr Coeff
As	74.922	Linear Thru Zero	1305.901369	0.000	0.999754
Cd	110.904	Linear Thru Zero	1740.109343	0.000	0.999981
Pb	207.977	Linear Thru Zero	16835.846092	0.000	0.999821



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# Quantitative Analysis - Summary Report

## Sample ID: 1

Sample Date/Time: Wednesday, March 28, 2012 12:18:23

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)1.011

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	142515	1.392	216.003	7.911
Cd	111	1647	2.731	113.334	14.264
Pb	208	44503	3.153	200.002	10.149

### Concentration Results

Analyte	Mass	Net Intens. Mean	Conc. Mean	Conc. SD	Conc. RSD	Sample Unit
As	75	142299.199	108.966	1.52	1.4	ppb
Cd	111	1533.482	0.881	0.03	2.9	ppb
Pb	208	44302.731	2.631	0.08	3.2	ppb

# Quantitative Analysis - Summary Report

## Sample ID: 2

Sample Date/Time: Wednesday, March 28, 2012 12:20:07

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)\2.012

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		157499		0.674	216.003		7.911
Cd	111		1575		1.472	113.334		14.264
Pb	208		43551		1.798	200.002		10.149

### Concentration Results

Analyte	Mass	Net Intens.	Mean	Conc. Mean	Conc. SD	Conc. RSD	Sample Unit
As	75	157283.319		120.440	0.81	0.7	ppb
Cd	111	1462.136		0.840	0.01	1.6	ppb
Pb	208	43350.754		2.575	0.05	1.8	ppb

# Quantitative Analysis - Summary Report

## Sample ID: 3

Sample Date/Time: Wednesday, March 28, 2012 12:21:52

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)\3.013

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
s	75	212719	2.242	216.003	7.911
d	111	1473	6.087	113.334	14.264
b	208	307255	2.932	200.002	10.149

### Concentration Results

Analyte	Mass	Net Intens. Mean	Conc. Mean	Conc. SD	Conc. RSD	Sample Unit
s	75	212503.415	162.725	3.65	2.2	ppb
d	111	1360.119	0.782	0.05	6.6	ppb
b	208	307054.847	18.238	0.54	2.9	ppb

# Quantitative Analysis - Summary Report

## Sample ID: 4

Sample Date/Time: Wednesday, March 28, 2012 12:23:37  
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)4.014  
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		234419		3.262	216.003		7.911
Cd	111		1521		4.148	113.334		14.264
Pb	208		305874		3.700	200.002		10.149

### Concentration Results

Analyte	Mass	Net Intens.	Mean	Conc. Mean	Conc. SD	Conc. RSD	Sample Unit
As	75		234202.620	179.342	5.86	3.3	ppb
Cd	111		1407.460	0.809	0.04	4.5	ppb
Pb	208		305673.729	18.156	0.67	3.7	ppb

# Quantitative Analysis - Summary Report

Sample ID: 5

Sample Date/Time: Wednesday, March 28, 2012 12:25:22

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)\5.015

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
s	75		239506		3.026	216.003		7.911
d	111		4815		2.694	113.334		14.264
b	208		59681		4.070	200.002		10.149

### Concentration Results

Analyte	Mass	Net Intens.	Mean	Conc. Mean	Conc. SD	Conc. RSD	Sample Unit
s	75		239290.481	183.238	5.55	3.0	ppb
d	111		4701.942	2.702	0.07	2.8	ppb
b	208		59481.474	3.533	0.14	4.1	ppb

# Quantitative Analysis - Summary Report

## Sample ID: 6

Sample Date/Time: Wednesday, March 28, 2012 12:27:08

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)6.016

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		241281		1.698	216.003		7.911
Cd	111		4793		2.841	113.334		14.264
Pb	208		59743		4.014	200.002		10.149

### Concentration Results

Analyte	Mass	Net Intens.	Mean	Conc. Mean	Conc. SD	Conc. RSD	Sample Unit
As	75		241064.552	184.596	3.14	1.7	ppb
Cd	111		4679.263	2.689	0.08	2.9	ppb
Pb	208		59542.535	3.537	0.14	4.0	ppb

# Quantitative Analysis - Summary Report

## Sample ID: 7

Sample Date/Time: Wednesday, March 28, 2012 12:28:53

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)\7.017

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	180575	0.359	216.003	7.911
Cd	111	559	1.256	113.334	14.264
Pb	208	162256	3.207	200.002	10.149

### Concentration Results

Analyte	Mass	Net Intens. Mean	Conc. Mean	Conc. SD	Conc. RSD	Sample Unit
As	75	180359.115	138.111	0.50	0.4	ppb
Cd	111	446.016	0.256	0.00	1.6	ppb
Pb	208	162056.145	9.626	0.31	3.2	ppb

## Quantitative Analysis - Summary Report

### Sample ID: 8

Sample Date/Time: Wednesday, March 28, 2012 12:30:38

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)\8.018

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		178993		1.489	216.003		7.911
Cd	111		612		7.850	113.334		14.264
Pb	208		171096		0.671	200.002		10.149

#### Concentration Results

Analyte	Mass	Net Intens.	Mean	Conc. Mean	Conc. SD	Conc. RSD	Sample Unit
As	75		178776.513	136.899	2.04	1.5	ppb
Cd	111		498.687	0.287	0.03	9.6	ppb
Pb	208		170896.438	10.151	0.07	0.7	ppb

# Quantitative Analysis - Summary Report

## Sample ID: 9

Sample Date/Time: Wednesday, March 28, 2012 12:32:20  
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)\9.019  
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
s	75	223178	0.432	216.003	7.911
d	111	3403	2.503	113.334	14.264
b	208	76875	3.398	200.002	10.149

### Concentration Results

Analyte	Mass	Net Intens. Mean	Conc. Mean	Conc. SD	Conc. RSD	Sample Unit
s	75	222961.598	170.734	0.74	0.4	ppb
d	111	3289.303	1.890	0.05	2.6	ppb
b	208	76674.577	4.554	0.16	3.4	ppb

# Quantitative Analysis - Summary Report

## Sample ID: 10

Sample Date/Time: Wednesday, March 28, 2012 12:34:03

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)\10.020

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		225571		1.557	216.003		7.911
Cd	111		3450		3.339	113.334		14.264
Pb	208		79665		5.662	200.002		10.149

### Concentration Results

Analyte	Mass	Net Intens.	Mean	Conc. Mean	Conc. SD	Conc. RSD	Sample Unit
As	75		225354.656	172.566	2.69	1.6	ppb
Cd	111		3336.654	1.917	0.07	3.5	ppb
Pb	208		79464.936	4.720	0.27	5.7	ppb

The image features a large, faint watermark of the Universiti Pendidikan Malaysia (UPM) logo in the background. The logo is a shield-shaped emblem with a red and white color scheme. It contains the letters 'UPM' in a red box at the top left, an open book in the center, and a stylized figure or symbol below. The text 'APPENDIX II' is centered over the logo.

# **APPENDIX II**

## Appendix II



Figure 1.1: *Geragau* shrimp



Figure 3.1: Study location, Kg. Pinang, Melaka.

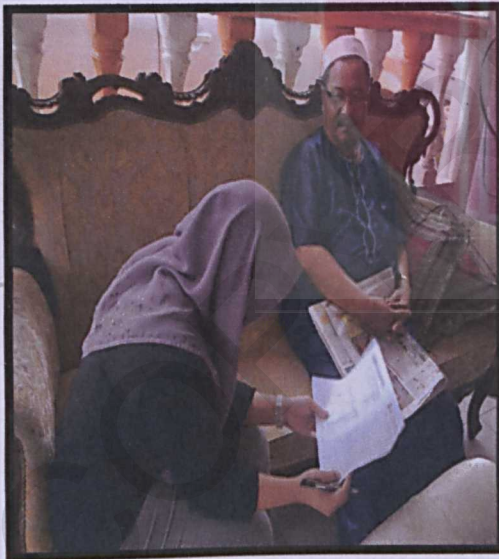


Figure 3.12: Interviewed was done to the respondents



Figure 3.13: Body weight of respondent was measured during data collection.

# APPENDIX III

### Appendix III: Dry Ashing Method



Figure 3.6: Shrimp paste sample

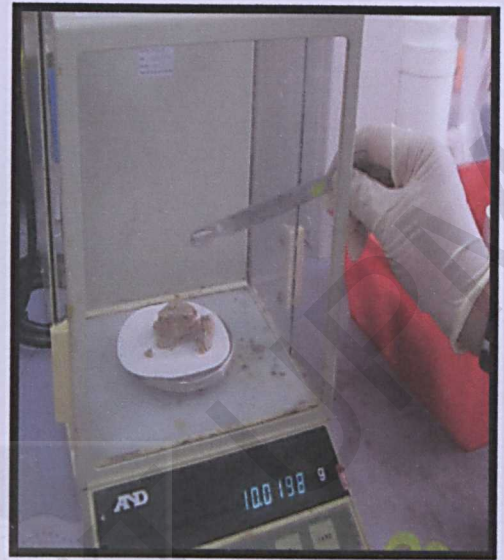


Figure 3.7: One gram of sample was weighted and placed in a crucible.



Figure 3.9: Sample were removed from the furnace and cooled down; 2 ml of 5 M  $\text{HNO}_3$  was added and evaporated to dryness on a water bath.



Figure 3.8: Sample was placed in a preheated muffle furnace at 200–250 °C for 30 min. Then, sample was ashed for 4 h at 480 °C. 2 ml of concentrated HCl was added and the sample was evaporated to dryness, removed. 5 ml of 2 M HCl was added and the tube was again swirled. Continued.



Figure 3.10: The solution was filtered through Whatman No. 42 filter paper and transferred quantitatively to a 25 ml volumetric flask by adding distilled water



Figure 3.11: The samples ready to be analyzed by using ICP-MS.

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Figure 3.10: The solution was filtered through Whatman No. 42 filter paper and transferred quantitatively to a 25 ml volumetric flask by adding distilled water



Figure 3.11: The samples ready to be analyzed by using ICP-MS.

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