UNIVERSITI PUTRA MALAYSIA

ARSENIC CONCENTRATION IN ANCHOVY SAUCE AND HEALTH RISK ASSESSMENT AMONG RESIDENTS IN TUMPAT, KELANTAN

BY
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ABSTRACT

ARSENIC CONCENTRATION IN ANCHOVY SAUCE AND HEALTH RISK ASSESSMENT AMONG RESIDENTS IN TUMPAT, KELANTAN

SUZILLA YAACOB

Introduction: Anchovy sauce is a Malaysian preserved sauce and one of the best known fermented seafood products in Kelantan, Malaysia. This food was contaminated with heavy metals caused by human activities which contaminate the main source of this food. **Objectives:** This research was conducted to determine the arsenic concentration in anchovy sauce, and to assess the health risk and determine the prevalence of acute and chronic arsenic poisoning sign among respondents. **Materials and method:** A total of 160 of respondents were recruited from Kg Pengkalan Kubur, Tumpat based on inclusion criteria. Three brands of anchovy sauce samples were purchased from local market based on response from food frequency questionnaire. Arsenic concentration in anchovy sauce samples were determined by dry ashing methods and analyzed with Inductively Coupled Plasma Mass Spectrometer (ICP-MS).**Results:** Arsenic concentration in anchovy sauce samples was 2.807 mg/kg and exceeds the value permitted by Food Regulation 1985 which is 1 mg/kg. Based on the health risk assessment results, there is a significant relationship between the frequent consumption of anchovy sauce and the lifetime cancer risk and hazard quotient which is $p < 0.0001$ encountered by villagers. However, the health risk assessment showed HQ and LCR level not exceed 1 which indicate acceptable level which respondents might not manifested the health effect yet. **Conclusion:** Arsenic was detected in anchovy sauce in this study which indicates that our food source might be contaminated with Arsenic. Public should aware in consuming these food because concern of it might cause the chronic diseases if consumed in continuous exposure. Manufacturer should improved food hygienic during food preparation to avoid food to be contaminated.

Keywords: *Arsenic concentration, Anchovy Sauce, Risk Assessment, Kelantan*

ABSTRAK

KAJIAN KEPEKATAN LOGAM BERAT ARSENIK DAN RISIKO KESIHATAN DIKALANGAN PENDUDUK DI TUMPAT, KELANTAN

SUZILLA YAACOB

Pengenalan: Budu adalah salah satu daripada produk makanan laut yang diperam dan terkenal di Kelantan, Malaysia. Makanan ini tercemar dengan logam berat disebabkan oleh pelbagai aktiviti manusia yang turut mencemari bahan asas produk ini. **Objektif:** Kajian ini telah dijalankan untuk menentukan kepekatan arsenik dalam budu dan untuk menilai risiko kesihatan dan juga menentukan prevalens arsenik akut dan kronik keracunan di kalangan responden.**Kaedah dan bahan:** Sebanyak seratus enam puluh orang responden telah diambil dari Kg Pengkalan Kubur, Tumpat dan tiga jenama sampel (budu) yang dibeli dari pasaran berdasarkan maklum balas daripada borang soal selidik. Arsenik dalam sampel budu ditentukan oleh pengabuan kering dan dianalisis dengan ICP-MS. **Hasil kajian:** Kepekatan arsenik dalam sampel budu adalah 2.807 mg / kg dan ia melebihi nilai yang dibenarkan oleh Peraturan Makanan 1985 iaitu 1 mg /kg. Berdasarkan keputusan penilaian risiko kesihatan, terdapat hubungan yang signifikan antara kekerapan pengambilan budu dengan LCR dan HQ yang mana p <0.0001.Walau bagaimanapun, penilaian risiko kesihatan menunjukkan HQ dan LCR tidak melebihi nilai 1 dan berada pada tahap yang boleh diterima yang mana kesan kesihatan terhadap responden tidak dapat dilihat dengan jelas lagi. **Kesimpulan:** Melalui kajian ini, Arsenik telah dikesan dalam budu yang menunjukkan bahawa sumber makanan mungkin dicemari oleh Arsenik. Orang ramai perlu mempunyai kesedaran dalam pengambilan makanan ini kerana kebimbangan ia mungkin menyebabkan penyakit kronik jika diambil secara berterusan. Pengeluar juga harus meningkatkan lagi tahap kebersihan terutamanya semasa proses pembuatan makanan ini untuk mengelakkannya daripada tercemar.

Keywords: *Kepekatan Arsenic, Budu, Penilaian Risiko, Kelantan*

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LIST OF ACRONYMS AND ABBREVIATIONS

As	Arsenic
ADD	Average daily dose
LADD	Lifetime average daily dose
ED	Exposure duration
EF	Exposure frequency
ATDSR	Agency for Toxic Substances and Disease Registry
ICP-MS	Inductively Coupled Plasma Mass Spectrometer (ICP-MS)
µg/ml	Microgram per mililiter
mg/kg	Milligram per kilogram
JPM	Jabatan Perangkaan Malaysia
RfD	Reference Dose
HQ	Hazard Quotient
LCR	Lifetime Cancer Risk
PTWI	Provisional Tolerable Weekly Intake
EPA	Environmental Protection Agency
USEPA	United States Environmental Protection Agency
WHO	World Health Organization
IRIS	Integrated Risk Information System

CHAPTER 1

INTRODUCTION

1.1 Background

Anchovy sauce or '*budu'*, is a Malaysian preserved sauce and one of the best known fermented seafood products of Kelantan in Malaysia. It was consumed mainly by people in the East Coast states of West Malaysia, namely Kelantan and Terengganu; and the Southern Provinces of Thailand (Rosma *et al.,* 2009). It is a common local dipping condiment served with mixed of lime, chillies and slices of onion. It could also be mixed with some other ingredients as a dipping condiment known as '*sambal belacan'* which well liked by Malaysians especially in North area (Ghazali, 2011). Almost half of the family in that state has at least one bottle of anchovy sauce at their kitchen. Recently, '*budu*', Kelantan fish sauce has become popular among other consumer in Malaysia. This food said to have many benefits in terms of health. Recent discoveries from nutrition experts of Japan, United States, Australia, New Zealand and Germany prove anchovy sauce content has glutathione as antioxidant, produce toxic waste and improve the immune system (immune) that can fight against various fatal diseases and prevent cancer (Kato *et al.,* 2004). Other than that, KOSMO newspaper online reported that the research from Senior Research Environment and Bioprocess Technology Centre SIRIM, Hamidah Sidek discover anchovy sauce as a skin lightening and anti-aging agent.

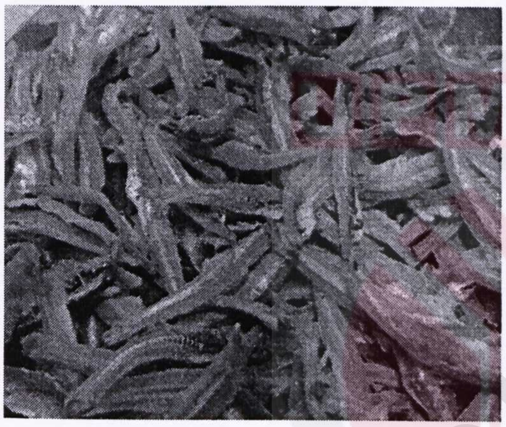

Figure 1: Dried anchovies

Figure 2: Sample of anchovy sauces

Anchovy sauce made from anchovy, salt and sugar as shown in Figure 3 (Appendix 1). It is traditionally made by mixing anchovy and salt in the certain range of ratio and allows fermenting for six months. Basically, anchovy sauce process activity begins during June to November because it is anchovy season. During this time, cleansing process and separation process for anchovy was made by workers at river banks before it bring to the seaside. Anchovy was fermented in barrel glass. The usage of glass barrel was suggested by SIRIM in order to increase the cleanliness of product.

While, during December to May is the time to cook, filter and pack the product. After anchovy sauce was cooked, filtered and cooled for a few hours then it was packaged and bottled with the machine and man-made as in Figure 2.

Anchovy's sauce served as local raw food in our Malaysian cookings besides salted fishes, dried shrimps and shrimp paste. It goes well with starters and excellent with plain white rice and is a must with fried rice dishes like *nasi goreng.* It is also used as a flavoring and is normally taken with fish, rice and raw vegetables.

Frequent consumption of anchovy sauce may lead into the accumulation of several of heavy metal in human body. Heavy metals have long been recognized as an important pollutant due to their toxicity and ability to accumulate in marine organisms. A previous study by Suhaimi, 2006 that was conducted on fish, heavy metal found such on arsenic was detected in low amount. However, the presence of Arsenic was concern enough into our human food processing if the exposure is continuous. As can exist in anchovy sauce during food processing and also from its origin sources such as anchovy and small fish like *Stolephorus spp.,and Sardinella spp.* as shown in Figure 1 (Rosma *et al.*,2009). Dried anchovies and small fish were comes from local deep sea which has been contaminated with marine pollution. As proven in the previous study, As concentration in anchovy were 0.023 ± 0.01 mg/kg (Naransal, 2001). Nevertheless, the value was not exceeded the Food Regulation 1985 but it is prove the As exist in marine lives.

Such contamination may be due to a high level of local environmental contamination, for example from a local marine. Fish might be exposed to As in polluted and contaminated waters. Hence, it gives big impacts to the human dietary habit as absorption of small amounts of arsenic may cause serious effects on human health and affecting the body in several ways. Health effect of arsenic can be seen in acute and chronic symptoms. Symptoms of acute toxicity include severe vomiting and diarrhea accompanied by muscular cramps, facial oedema, nervous and sensory loss. Chronic exposure to arsenic compounds is associated with skin lesions and hyperkeratosis (FEHD, 2002). Carcinogenicity in the skin, lung, bladder, kidney and liver is strongly supported by epidemiological studies in China that showed a correlation between fish sauce and the incidence of cancer (Cai, 2000).

1.2 Problem statement

Anchovy sauce are widely consumed and commonly served in everyday serving. Since this food product are popular to serve with other dishes, the safety of this food product into our health is quite concern. From the previous studies which are the study about the toxicity evaluation into our locally raw food products include fish products shows that it contains several heavy metals such as cadmium, lead, mercury and arsenic. People as consumers of anchovy sauce may be affected by consuming them as it is also originate from fish sources. It may cause long term effects and must be consumed in large amount and frequent.

Marine fishery products may contain high levels of arsenic as edible muscle fish tissues are as a site of high arsenic accumulation (Jankong et *al.,* 2007). Fish may accumulate heavy metals and represent one of the major sources of heavy metals for human. Therefore, the determination of the heavy metals burden in commercial seafood can provide useful information in ensuring the safety of the food supply and also minimizing human exposure (Ashraf, 2006).

1.3 Study justification

This research was conducted to determine the contamination level of arsenic in the anchovy sauce and to estimate the risks to human health associated with frequent consumption of this food. The aim of this study was to provide information on the arsenic levels in the anchovy sauce. Anchovy sauce was the food of choice because the main ingredients to make anchovy sauce which is abundantly available in our local marine. According to that, there are some studies by Francesconi, & Edmonds that focused on the determination of arsenic in marine organisms such as fish, marine algae, seagrass, oyster and other organisms, primarily because these are major sources of arsenic entering the human diet (Francesconi and Edmonds, 1997).

In addition, the concentrations of arsenic in anchovy sauce will be compared to the Food Regulation 1985 to determine the status of arsenic exposure per day in the individual diet. This research also to estimate how much the quantity of arsenic ingest by the people. Estimation made by knowing how much anchovy sauce was consumed by the community. This is because arsenic that contain in this food may have implications on human health.

Ingestion of some heavy metal may occur in people who have high intake of these foods. It is well known that ingestion of contaminants in excessive amounts can have detrimental effects on health (Lowik, 1996). However, there have not been many studies conducted to estimate the content of heavy metals in our local processed food products. So that, this research was conducted in order to determine and estimate the health risk to people who consumed anchovy sauce. Data and publications on heavy metal contamination especially arsenic in our local food products is limited because a study of it not much.

The importance of this study was to determine the consumption of anchovy sauce among community in Kg Pengkalan Kubur in Tumpat, Kelantan whether they like very much to consume anchovy sauce with their meal as Tumpat is a main place to process anchovy sauce. The high concern of anchovy sauce usage among population must be determined in order to know the recent dietary intakes of arsenic in their food processing. A survey on the distribution of arsenic in consumable anchovy sauce in this district was needed. Moreover, there are a data from Jabatan Alam Sekitar (JAS) and Jabatan Perangkaan Malaysia (2000) of the marine pollution regarding with the arsenic concentration on the east coastal peninsular Malaysia. The data from 1999-2004 by JAS stated that arsenic level in east coastal 0.002 μg/L. Therefore, this study was designed to determine the concentrations of arsenic in anchovy sauce that they served with meal.

1.4 Conceptual framework

Figure 4 (Appendix 2) showed the conceptual framework of this study which is highlighted the area of the study and how the community would get the Arsenic content via anchovy sauce intake. People exposed to the arsenic through three sources which are the occupational exposure, environmental exposure and dietary intake. However, the focus on this study is on the food intake (anchovy sauce) which will affect the community of Kg Pengkalan Kubur.

Community will be exposed to the arsenic contamination *via* ingestion. Ingestion is the main route for arsenic exposure. Due to the arsenic exposure, human will be affected through non carcinogenic effects and carcinogenic effects. For non carcinogenic effects the disease normally can be found such as dermatitis, hypersensitivity and allergic reaction. While carcinogenic effects usually focus on cancer of bladder, lung, kidney and skin.

1.5 Definition

1.5.1 Arsenic concentration in anchovy sauce

Conceptual

Arsenic concentration is the amount of arsenic which can be detected in anchovy sauce sample. The acceptable standard level for As detection in this samples given by Food Regulation 1985 is 1 mg/kg.

Operational

The concentration of Arsenic in anchovy sauce was measured using Inductively Coupled Plasma Mass Spectrometer (ICP-MS) ELAN DRC-e. The unit is in µg/L.

1.5.2 Health risk assessment

Conceptual

Risk assessment is a process in which information is analyzed to determine if a hazard might cause adverse effects to humans following exposure under defined condition to a risk source. Risk assessment consists of four steps, namely hazard identification, dose response assessment, exposure assessment and risk characterization (Saipan, 2009).

Operational

Health risk assessment is risk assessment applied for the assessment of health hazards from the environment which determine the carcinogens and non-carcinogens and different scale for carcinogen and non-carcinogen. Health risk assessment can be into respondents by using food frequency questionnaire and also take into account the arsenic concentration in food sample.

1.6 Research objectives

1.6.1 General objective

To determine the arsenic concentration in anchovy sauce and health risk among residents in Tumpat.

1.6.2 Specific objectives

a) To determine the frequency of anchovy sauce consumption among respondent
b) To determine arsenic concentration in anchovy sauce.
c) To determine the prevalence of acute and chronic arsenic poisoning signs of villagers.

d) To determine the health risk of respondents indicated by lifetime average daily dose (LADD), average daily dose (ADD), health quotient (HQ) and lifetime cancer risk (LCR).

e) To determine the relationship between the frequencies of anchovy sauce consumption and the prevalence of acute and chronic arsenic poisoning signs among villagers.

f) To determine the relationship between the frequency of anchovy sauce consumption and health risk among respondents.

1.7 Study hypothesis

1. There is a significant association between the frequencies of anchovy sauce consumption and the prevalence of acute and chronic arsenic poisoning signs among villagers.

2. There is a significant relationship between the frequencies of anchovy sauce consumption and health risk among respondents.

CHAPTER 2

LITERATURE REVIEW

2.1 Arsenic

Arsenic is a metalloid; a natural element that is not actually a metal but has some properties of metal. It is a natural component of the Earth's crust, generally found in trace quantities in all rock, soil, water and air. Based on the Suhaimi research in (2006), arsenic and its compounds are widely distributed in nature primarily in two oxidation states, arsenite (trivalent) and arsenate (pentavalent). Arsenite and arsenate in other terms is inorganic arsenic which is can be found in water. It can exist in many different chemical forms in combination with other elements. Some forms are inorganic, which do not contain carbon, and others are organic, which always contain carbon. Some other in organic form which is usually exists in food.

2.2 Arsenic in environment

2.2.1 Natural sources

Arsenic enters the environment from both natural and anthropogenic sources. The Earth's crust is an abundant natural source of arsenic. About one-third of the arsenic in the Earth's atmosphere is of natural origin. Volcanic action is the most important natural source. Inorganic arsenic of geological origin is found in groundwater used as drinking water. Arsenic occurs extensively in the environment in different chemical species especially in natural water systems where it generally occurs in the oxidation states As (III) and As (V) as well as in food chain in various other forms (Yusof *et al.,* 1994). It has become one of the most widely measured trace metals in environmental programs due to its toxicity and possible carcinogenic effect. Principally, arsenic occurs in natural waters as arsenate As (V) and lesser extent as arsenite As (III). Arsenate will be more toxic in waters deplete with dissolved oxygen and at lower pH.

2.2.2 Man-made sources

In the Asian region, arsenic is traceable to residuals from mining activities and geothermal energy generation (Deocadiz, 1999). Mining, smelting of non-ferrous metals and burning of fossil fuels are the major industrial processes that contribute to anthropogenic arsenic contamination of air, water and soil. Historically, use of arseniccontaining pesticides has left large tracts of agricultural land contaminated. The use of arsenic in the preservation of timber has also led to contamination of the environment.

Moreover, major sources of arsenic in surface waters of the ocean are riverine inputs carrying arsenic-contaminated drainage from the land, and upwelling of deep ocean water enriched in arsenic (Neff, 1997). Therefore, the concentration of arsenic is higher in estuaries and coastal water compared to the open sea. As in Malaysia there has a study on the coastal of peninsular Malaysia on the Arsenic compound. It is been said that the parameter of the marine pollution such as oil and grease, total solid suspended, bacteria (*E.coli*) and heavy metal including arsenic. This parameter was used to determine the level of marine pollution.

Arsenic is released into the environment by the smelting process of copper, zinc, and lead, as well as by the manufacturing of chemicals and glasses. Arsine gas is a common byproduct produced by the manufacturing of pesticides that contain arsenic. Arsenic may be also being found in water supplies worldwide, leading to exposure of shellfish. Other sources are paints, rat poisoning, fungicides, and wood preservatives.

2.3 Level arsenic in living organism

Marine organisms normally contain arsenic residues ranging from less than 1 to more than 100 mg/kg, predominantly as organic arsenic species in invertebrates and fish. Background arsenic concentrations in freshwater and terrestrial biota are usually less than 1 mg/kg. Humans may be exposed to arsenic through food, water and air. The concentration of arsenic in seafood product is varying. It is depends on the where seafood is manufactured, the sources of the seafood etc. As anchovy sauce was made from anchovies, so that marine fishes could be consider as one of the sources of contamination. It is proved in the one of the study related with arsenic contamination in marine fishery. From the study, it was stated that marine fishery products may contain high levels of arsenic as edible muscle fish tissues are as a site of high arsenic accumulation and significant amounts of inorganic arsenic in the muscle tissue. (Jankong *et al.,* 2007).

The other study also shows that the amount of arsenic present in some fish is greater than had been supposed. In recent years, numerous studies have focused on the determination of arsenic in marine organisms, such as fish, marine algae, sea grass, oyster and other organisms, primarily because these are major sources of arsenic entering the human diet (Francesconi and Edmonds, 1997).

2.4 Level of arsenic for human exposure

For human exposure to arsenic in the environment is primarily through the ingestion of food and water. Of these, food is generally the principal contributor to the daily intake of total arsenic. In some areas arsenic in drinking water is a significant source of exposure to inorganic arsenic. Food is a major pathway of arsenic intake for the general adult population, contributing more than 80-85% of the total daily intake. From the previous studies by the Lin and Liao, arsenic as a ubiquitous metalloid is one of the most toxic elements for human and animal health that cause toxic and detrimental biological effects such as different kinds of cancer. Studies on total diet arsenic in different countries have indicated that fish is one of the most significant dietary dishes in this respect (Lin and Liao, 2008).

Following to the research from Naransal (2001), the study on the heavy metal content in raw food at Pasar Semenyih reported that arsenic does not exist in raw food except in seafood. Due to the fact that even trace amounts of some heavy metals can generally exhibit high toxicity to marine biota and human, there is an increasing interest in studying these metals in the marine environments. These heavy metals represent the greatest potential concern to the environment and human health. Heavy metal accumulates on available surfaces such as plants and aquatic bodies or sediments; they can be magnified through the food chain, thereby posing health risk to humans who consume sea food from contaminated sources (Shafie *et al.,* 2006). In the present study, a preliminary survey on heavy metals was conducted on nine species of commonly consumed fish and shellfish by Suhaimi (2006). The heavy metals investigated include cadmium, lead, arsenic and mercury because they are known to accumulate in the environment and in fish, and are known to cause adverse health effects if consumed in sufficient quantities. Meanwhile ,the other study that was conducted in Korea by the Haeng *et al* (2006) on the food/dishes including food dishes that contain seafood also shows heavy metal exists in that food. From that study, arsenic was detected in concentrations average dietary intake of arsenic by the Korean population was 38.5 mg/person/day and 4.9 mg/kg body weight/week with ND to be on the safe side. Foods with rather high arsenic contents were mostly dried and broiled laver (seaweed) and salt-fermented and broiled fish.

2.5 Effects on human health

Arsenic is the most common cause of acute heavy metal poisoning in adults and is number 1 on the ATSDR's "Top 20 List."(ATSDR, 2007). Inorganic arsenic is considered carcinogenic and is related mainly to lung, kidney, bladder, and skin disorders (ATSDR, 2007). The International Agency for Research on Cancer (IARC) has assigned arsenic, inhaled or ingested, to Group I human carcinogens, because there is sufficient evidence from epidemiological studies to support a causal association between arsenic exposure and cancer (Tsuda *et al*., 1992). These effects have been demonstrated in many studies using different study designs. Inorganic arsenic is acutely toxic and intake of large quantities leads to gastrointestinal symptoms, severe disturbances of the cardiovascular and central nervous systems, and eventually death. Populations exposed to arsenic *via* drinking water show excess risk of mortality from lung, bladder and kidney cancer, the risk increasing with increasing exposure. There is also an increased risk of skin cancer and other skin lesions, such as hyperkeratosis and pigmentation changes (Jarup, 2003).

CHAPTER 3

METHODOLOGY

3.1 Study location

A cross sectional study was conducted among community in district of Tumpat, Kelantan. Kg Pengkalan Kubur was selected for this study. This location was chosen because it is main production and distribution centre for Malaysia. So that, there was possibility of the community at this area widely consumed the anchovy sauce. Hence, it can represent the anchovy sauce consumer in Kelantan.

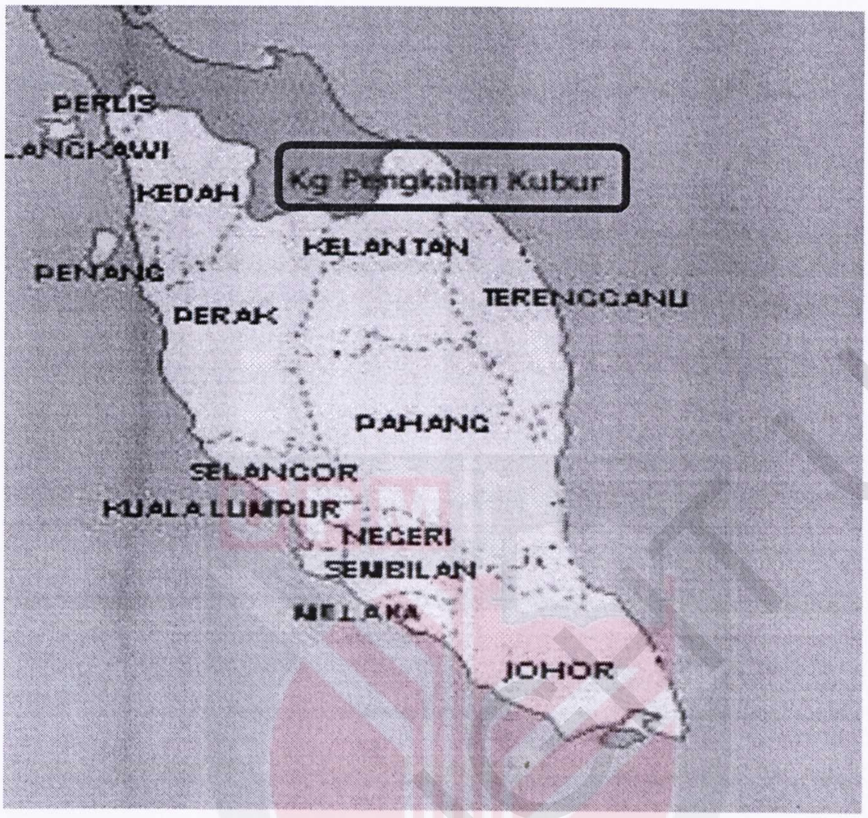

Figure 5: Location of sampling site

3.2 Study sample

Three samples of anchovy sauce were purposively purchased from local market and grocery stores from the study area based on the finding from the food frequency questionnaire. The food frequency questionnaire purpose was to determine the most popular anchovy sauce consumed by the respondents.

3.3 Study population

A total of 160 respondents randomly recruited based on (Neuman, 1997) calculation which fulfills the inclusive criteria. According to the Neuman (1997) the sample will be selected using the proportion formulae:

The population in the study area as the following:

- Kg.Pengkalan Kubur = 1584

As the population exceeds 1000 people, we used the moderately large population formulae which is about 10 % of population was yield. The calculation as follows:

$$\text{Kg. Pengkalan Kubur} = \frac{10}{100} \times 1584 = 158 \text{ respondents}$$

The total of the respondents for study area is 158 respondents.

3.3.1 Sampling method

Respondents were chosen from Kg.Pengkalan Kubur. It was selected through random sampling method from study area. Most of the respondents were selected in the range of 18 to 59 years old (Norimah *et al.*, 2008). It was included all family members in the house who consumed anchovy sauce on their dishes and lived in this village at least 1 year and above (Dzulfakar *et al.*,2011).

3.4 Study design

This cross-sectional study was conducted among community in Kg. Pengkalan Kubur, Tumpat. This study was carried out between September 2011 and May 2012 as demonstrated in Gannt chart in Table 18 (Appendix 3).

3.5 Data collection and instrumentation

The instruments that were used in this research which are questionnaires, Seca Body Weighing Scale, Seca Body Height Scale as shown in Figure 6 and Figure 7 and Inductively Coupled Plasma Mass Spectrometer (ICP-MS) ELAN DRC-e (ICP-MS) in Figure 8. The objective of the data collection was to know the arsenic exposure and arsenic concentration in anchovy sauce. The arsenic exposure were determined by using pre-tested structured questionnaire while arsenic concentration were measured by extraction process via dry ashing method with muffle furnace and Inductively Coupled Plasma Mass Spectrometer (ICP-MS) ELAN DRC-e for detection the sample and health risk assessment to know the Average Daily Dose (ADD), Lifetime Average Daily Dose (LADD), Lifetime Cancer Risk and Hazard Quotient (HQ).

3.5.1 Questionnaire

A set of pre-tested structured questionnaire were develop based on Malaysian Adult Nutrition Survey 2003 was used in this study which comprised of five sections.

Questions were developed according to the aim of the researchers. The first section contained questions regarding socio-demographic data such as age, household income, occupation, races and education level. The second section contained questions regarding on the health information of the respondent focus on the arsenic symptoms. The third section consists of anthropometry information of the respondents such as weight and height. The forth section is for food frequency intake by the respondents. The information of consumers' consumption of anchovy sauce in the selected village was asked in the food frequency questionnaire. It is include the frequency of anchovy sauce they used, the quantity of anchovy sauce they consumed and the sources of anchovy sauce will be asked in the food frequency questionnaire besides other food items as a confounder. Food frequency questionnaire will be focus on the food intake by the respondents. The frequency of the food intake among respondents was measured as the following:

1. How much in a day
2. How much in a week
3. How much in a month
4. How much in a year
5. Never

The range of food frequency questionnaire was then categorized into two categories which is less frequent and frequent for analysis. The last section of this questionnaire is regarding on the arsenic exposure from other sources such as drinking water sources, smoking habit and alcohol consumption, insecticide usage and exposure to the pesticide.

During the data collection period, the questionnaires then were distributed among respondents and the respondents were interviewed in person with the researcher at their respective houses to be increase the understanding of the people. An interview was conducted to collect all exposure factors.

3.5.2 Weight scale and height scale

In determining the LADD and ADD, body weight measurement was taken using a Seca Body Weighing Scale. While, the height of the respondents were measured using Seca Height Scale to know the nutritional status of the respondents.

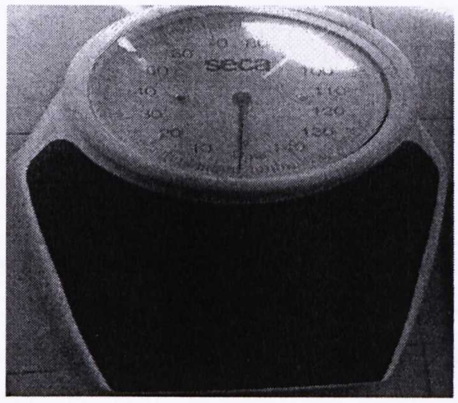

Figure 6: SECA Weighing Scale

SECA Weighing Scale (Source: SECA Website, 2011)

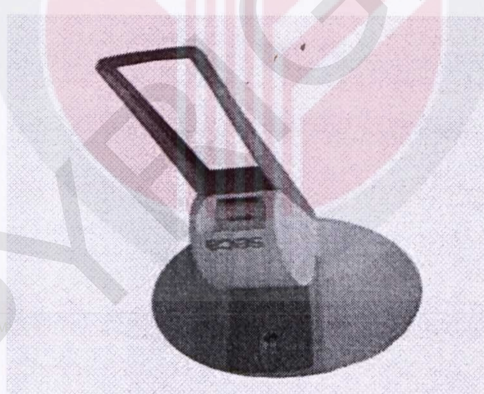

Figure 7: SECA Height Scale

SECA Height Scale (Source: SECA Website, 2011)

3.5.3 Inductively Coupled Plasma Mass Spectrometer (ICP-MS)

3.5.3.1 ICP-MS ELAN DRC-e

In this study, Perkin Elmer model ELAN DRC-e inductively coupled plasma-mass spectrometry (ICP-MS, USA).This instrument has established itself as a major technique for trace element determination in foodstuffs due to many desirable features such as low LOQs, multi-element capability, and wide linear dynamic range. The technique utilizes high-energy argon plasma (8000 K) to convert the sample constituents to their elemental components which are then ionized and transported to the mass spectrometer for selective detection and quantification. Arsenic, which has only one naturally occurring isotope, is measured at m/z 75 (As+). The ICP-MS was optimized according to the manufacturing standards. The table 1 shows the analytical conditions for determining the arsenic by the inductively coupled plasma mass spectrometry (ICP-MS).

3.5.3.2 Reagents

Prior to the experiments the apparatus were sterilized by soaking them overnight in diluted nitric acid (20%) and were later rinsed with deionised water. All the standard stock solutions were prepared with de-ionized water (18.2 MΩ cm) purified using a Milli-Q water purification system with multi-element calibration standard 3 ranges from 10 ppb ,30 ppb,50 ppb,100 ppb and blank solution which is from deionised water.

Table 1 ICP–MS Operating Conditions and Performance

Performance	Operating Conditions
ICP-MS	ELAN DRC-e
Nebulizer gas flow	0.72 L/min
Auxiliary gas flow	1.20 L/min
Plasma gas flow	17.00 L/min
ICP RF Power	1100 W
Reading/Replicates	3

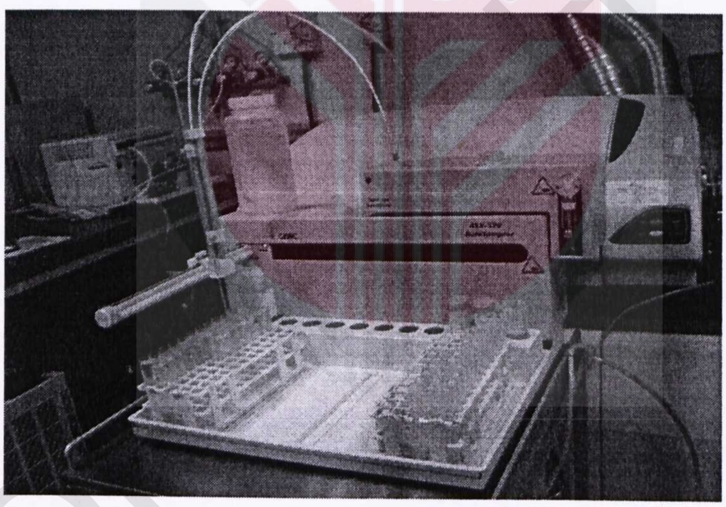

Figure 8 Inductively Coupled Plasma Mass Spectrometer (ICP-MS)

3.5.4 Sample analysis

3.5.4.1 Dry -ashing

In determination of arsenic concentration, the methods were used have been described by Issac and Kerber (1971) (Appendix 4). Briefly, an accurate weight (10 g) of the anchovy sauce sample was weighted on analytical balance. Then, ten gram of sample in a crucible was placed in a preheated muffle furnace (Figure 9) 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. After that, 20 ml of 5 M HNO_3 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, 20 ml of concentrated HCl was added and the sample was evaporated to dryness, removed, and then again 50 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 250 ml volumetric flask and filled up to the mark by deionized water

Table 2 Description of parameter used for arsenic concentration calculation

Parameter	Symbols	Units	Parameter characteristics
Concentration of metal in the extract	C	µg/ml	0.11695
Dilution factor	B	-	4
Volume of extract	A	ml	60
Weight of the sample	W	g	10

Figure 9 Muffle furnace

3.5.4.2 Health risk assessment

To calculate the health risk assessment associated with arsenic concentration in samples, it involves two calculations which are the average daily dose (ADD), which is used to assess the non-carcinogenic effects and the lifetime average daily dose (LADD) for carcinogenic effects. From ADD and LADD the HQ and LCR value can be derived. The calculation was based on United States Environmental Protection Agency (U.S.EPA) guidelines. Calculations for non-carcinogenic effects (a) and carcinogenic effects (b) as the following:

a) Non-Carcinogenic effects:

$$\text{Average Daily Dose (mg/kg-day) ADD} = \frac{C \times IR \times ED \times EF}{BW \times ATnc}$$

Non-carcinogenic risks are quantified by the calculation of a Hazard Qoutient (HQ). If the calculated HQ is equal to or less than 1, the non-carcinogenic effect due to exposure pathway is assumed to be negligible while HQ more than 1 suggests that there may be concern for non-cancer effects (Saipan, 2009). HQ is calculated as follows:

$$HQ = \frac{ADD}{RfD}$$

b) Carcinogenic effects:

$$\text{Lifetime Average Daily Dose (mg/kg-day) LADD} = \frac{C \times IR \times ED \times EF}{BW \times ATc}$$

For carcinogenic effects, lifetime cancer risk (LCR) is accepted in ranges 10^{-4} to 10^{-6}. (Saipan, 2009).Arsenic cancer slope factor (CSF) (mg/kg/day) = 1.5 mg/kg/day (IRIS, 2007).

$$LCR = LADD \times CSF$$

In estimating risk levels, the mean and maximum total arsenic concentrations, represented by C, were determined from the data from the anchovy sauce samples (mg/g). The exposure frequency (EF) is the exposure frequency (days/year); represents the average per-capita number of meals by the population based on a long term average contact rate (EPA, 1992). For all calculations, body weight (BW), exposure duration (ED) (years) and ATnc is the averaging time, non-carcinogen (ED x 365 days/year); ATc is the averaging time for cancer effects, equal to the life expectancy time (70 x 365 = 25,550 days) (Saipan, 2009). Exposure parameters were evaluated from interviewed data. The principal exposure factors that have been taken into account to carry out the risk assessment calculations are presented in Table 12.

3.5.5 Assessment of exposure to arsenic due to consumption

The Provisional Tolerable Weekly Intake (PTWI) value is an estimate of the amount of a contaminant that can be ingested over a lifetime without appreciable risk. PTWI is established by the Joint Food and Agricultural Organisation for the United Nations (FAO) / World Health Organisation (WHO) Expert Committee on Food Additives (JECFA). PTWI values were used in this study to serve as reference values for safe levels of exposure to arsenic. The PTWI of arsenic in food is 0.015 mg/kg bw (equivalent to 2.1×10^{-3} mg/kg bw per day). To have a reference value on a daily basis, the PTWI was divided into 7 days, giving the provisional tolerable daily intake (PTDI) 2.1 mg/kg body weight day (WHO, 2010).

3.6 Quality control

To make sure all the procedure and study was properly done; the questionnaire was validated by performing a pre-test on 20 respondents in Kg Raja, Besut in Terengganu. The questionnaire had reliability alpha coefficient value of 0.79 from pre-test result.

The purpose of conducting pre-test was to make sure the questionnaire was appropriate to be asked to the respondents. Each of the samples was duplicated during sample analysis and the reading value was triplicate to get the reliable data. It is necessary for the analytical instruments (ICP-MS) to meet the standard before it can produce a reliable data. The calibration curve of arsenic in this study has coefficient $r^2 = 0.999$.

3.7 Statistical analysis

Data collected from the questionnaire including socio-demographic data, arsenic concentration in anchovy sauce, body weight of respondents, LADD, ADD, LCR and HQ of both study areas were analyzed using Statistical Package for Social Science (SPSS) version 19.0. While, Kolmogorov-Smirnov, Shapiro Wilk and Skewness test were used to determine the normality distribution of the variables. The data were then analyzed in two stages of analysis. The first stage was univariate analysis which is to measure the mean, median, standard deviation and mode value data of the respondents. Bivariate analysis was then used to test the hypothesis, which was divided into testing for statistical significant difference and testing for relationship. Statistical significance was defined as $p < 0.05$.

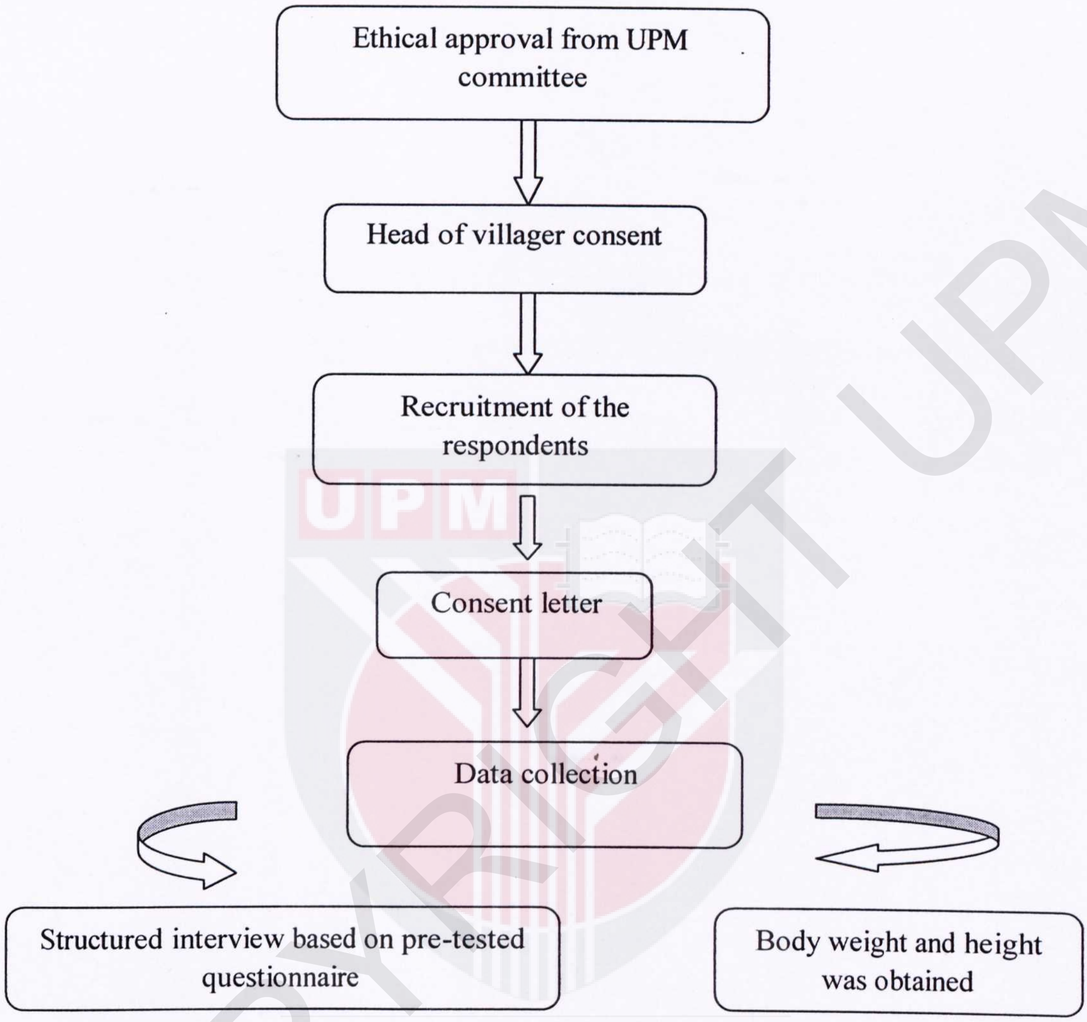

Figure 10 Data collection flow

3.8 Ethical consents

Prior to the data collection, approval was obtained from the Medical Research Ethics Committee and also from the head village for the questionnaire. All information about the respondents was confidential and it will not be shared with third parties and also written consent was obtained from the respondents prior to the data collection. Written consent was signed before data collection commenced.

CHAPTER 4

RESULTS

4.1 Socio-demographic information of the respondents

A sixty and hundreds respondent from study area were selected based on random sampling. Essential information including age, race, gender, household income and respondent body mass index were collected as shown in Table 3. The proportions of male and female in this study showed female were higher than male. Male and female made up 38 % and 62 % of the respondents. All respondents were Malays and Muslims. The highest categories for employment among respondents were in other category which contributed to 39 %.

Table 3 Socio-demographic information of the respondents (N=160)

Variables	n	%	Mean ± S.D	Median (IQR)	Range
Age (year) :					
18-29	70				
30-39	24	35 ± 12	34 (6)	18 – 58	35 ± 12
40-49	40				
50-59	26				
Gender:					
Male	61	38			
Female	99	62			
Body mass index :					
< 18.5	16	10			
18.5 – 24.9	100	62	23 ± 4	23(5)	16-38
25 – 29.9	33	21			
≥ 30	1	7			
Employment:					
Government	8	5			
Private	19	12			
Self-employed	36	23			
Unemployed	34	21			
Others	63	39			
Income per month (RM) :					
< 720	124	78		700(0)	0-3000
≥ 720	36	23			
Household					
<6	83	52	6 ± 2	5(3)	2-13
≥ 6	77	48			

* N=160

With regard to age, the majority were in range 18-29 years old (44%).The second highest was in 40-49 years old (25%). The least age of the respondents was in range 30-39 (15%) and 50-59 (16%) respectively. The mean and standard deviation for the age of the respondents was 35 ± 12.The distribution by BMI status based on WHO (1998) cut-offs were 10% underweight, 63% normal weight, 21% overweight and 7% obese. Mean and standard deviation for the body mass index were 23 ± 4.

Majority of the respondents (78%) had monthly income of less than 720 Malaysian Ringgit. The overall mean of household size was 6 and the median household size was 5. Household size less than 6 was 52 % while household size more than 6 was 48%.

4.2 Food frequency

Respondent's consumption pattern in anchovy sauce was illustrated in Figure 11. All respondents consume anchovy sauce. Based on the figure, the frequent consumption of anchovy sauce was indicated by everyday and 2-6 times per week. Majority of the respondents (60%) consumed anchovy sauce everyday and 2 to 6 times per week (34%). The others were in less frequent category.

Table 4 Frequency of anchovy sauce consumed by respondents

Frequency	N	%
Everyday	95	60
2-6 times a week	54	34
Once per week	2	1
Once per month	2	1
2-3 times a month	6	4
Seldom	1	1
Not consume	0	0

*N =160

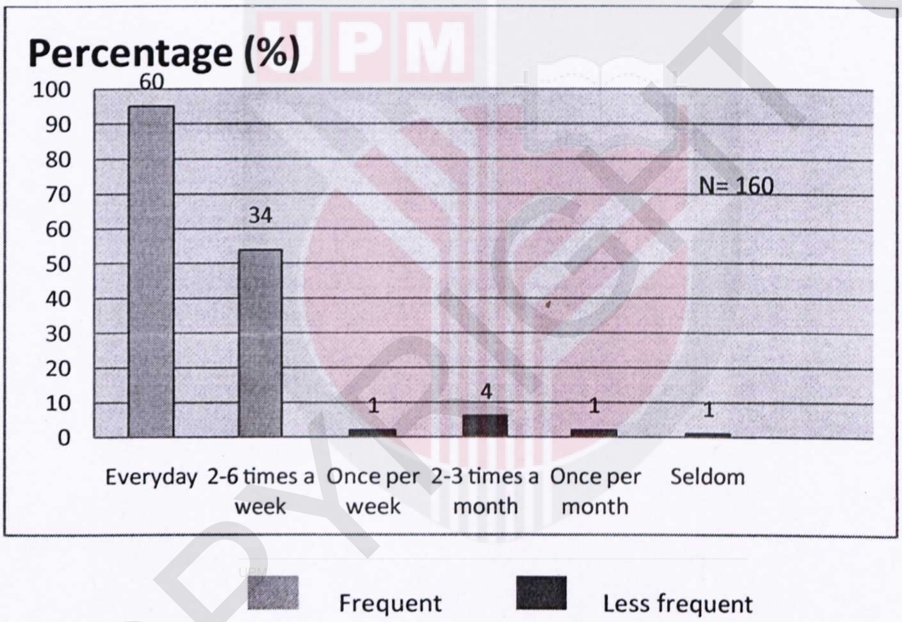

Figure 11 Bar chart of anchovy sauce frequency intake

Table 5 showed the prevalence and mean frequency of the most daily consumed foods among Kg Pengkalan Kubur residents. Generally, rice (100%), leafy vegetables (80%), and marine fish (84%) were eaten by the majority of the respondents in high proportion.

Majority of the respondents consumed rice everyday on average 1 plates of rice per day. Leafy vegetables and marine fish were consumed in average 1 cup and 1 piece respectively. Other seven food items eaten daily but by a smaller proportion of the respondents such as freshwater fish, cabbage ,bean vegetable, anchovy, shrimp paste, shrimp and canned fish. All these latter food items were consumed at least once a day and will contribute to other arsenic intake. The other table showed the most weekly intake of food consumed by respondents. Soy sauce (56.9%) and chillies/tomato sauce (55.6%) were the most consumed food by respondents.The other food consumed in small proportion by respondents. Average 3 teaspoon and 3 tablespoon of soy sauce and chilies/tomato sauce were consumed by respondents weekly.

Table 5 Prevalence and mean frequency of most daily consumed foods

Types of food	**Prevalence who answered daily consumption (%)**	**Mean frequency per day**	**Total amount consumed daily**
Rice	100	2.22	1 plate
Leafy vegetable	80	1.21	1 cup
Cabbage	49.4	0.68	1 cup
Bean vegetable	13.4	0.19	1 cup
Marine fish	84.4	1.64	1 piece
Freshwater fish	9.4	0.13	1 piece
Anchovy	3.1	0.04	1 ½ tablespoons
Canned fish	0.6	0.01	1 piece
Shrimp	3.8	0.06	3 piece
Shrimp paste	24	0.39	1 ½ teaspoons

*N =160

Table 6 Prevalence and mean frequency of most weekly consumed foods

Types of food	Prevalence who answered weekly consumption (%)	Mean frequency per week	Total amount consumed weekly
Soy sauce	56.9	3.57	3 teaspoon
Chilies/tomato sauce	55.6	3.63	3 tablespoon
Dried fish	43.4	1.79	1 piece
Oyster sauce	41.2	2.70	2 teaspoon
Fish cracker	40.6	2.13	5 pieces
Shrimp paste	40.6	4.07	2 teaspoon
Anchovy	40	1.62	1 tablespoon
Prawn	38.1	1.66	5 pieces
Bean sprouts	34.4	2.05	1 cup
Cuttlefish	33.8	0.23	3 piece

*N =160

4.3 Arsenic concentration

Total As concentration (mg/kg) in the sample of anchovy sauce was given in Table 7. The As concentration detected in anchovy sauce samples was 2.865 mg/kg. The levels of As in anchovy sauce samples were above the acceptable standard level given by Food Regulation 1985 (1 mg/kg) (Table 8). Thus, the anchovy sauce samples studied was not safe for human consumption in terms of As content.

Table 7 Detection of arsenic on anchovy sauce samples by using ICP MS

Anchovy sauce samples	Reading 1 (µg/ml)	Reading 2 (µg/ml)	Mean (µg/ml)
Sample 1	0.119513	0.119271	0.119392
Sample 2	0.118319	0.119846	0.1190825
Sample 3	0.112772	0.112006	0.112389

*N=3

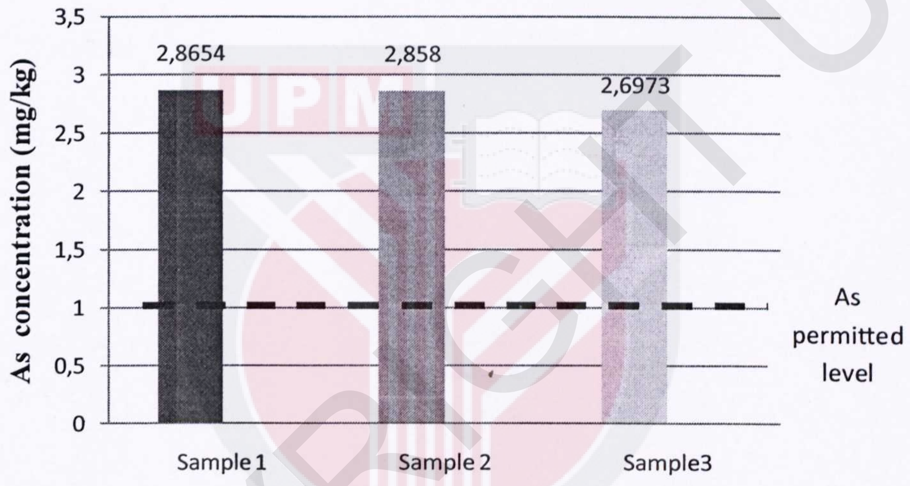

Figure 12 As concentration in anchovy sauce samples

Table 8 As concentration in Food Regulation 1985

Sample	Sample analysis values (mg/kg) daily	Food Regulation 1985 (mg/kg)
Anchovy sauce samples	2.807	1

4.4 Acute and chronic arsenic poisoning signs

Table 9 showed the prevalence of acute and chronic arsenic poisoning signs among respondents in Kg Pengkalan Kubur. From the table, headache (7%) was the most acute arsenic poisoning signs that faced by the respondents, following with fatigue (6%), allergic (5%) and muscle cramp (3%). While the other signs such as stomach ache, diarrhea and difficulty breathing have 1% respectively. There are no respondents who had vomiting symptoms.

Referring to the chronic arsenic poisoning signs table, numbness (9%) was the common sign among respondents followed by hair loss (3%) and the others were rashes and brittle nail (1%) respectively. None of the respondents were contracted by hyperkeratosis.

Table 9 Prevalence of acute and chronic arsenic poisoning signs

Acute signs	Yes		No	
	N	%	N	%
Stomach ache	1	1	159	99
Vomit	0	0	160	100
Diarrhoea	1	1	159	99
Muscle cramp	4	3	156	98
Headache	11	7	149	93
Difficulty breathing	1	1	159	99
Fatigue	9	6	151	94
Allergic	8	5	152	96
	Yes		No	
Chronic signs	N	%	N	%
Hyperkeratosis	0	0	160	100
Rashes	2	1	158	99
Hair loss	5	3	155	97
Brittle nail	1	1	159	99
Numb at hand	15	9	145	91

* N=160

4.5 Health risk evaluation

The median value of ADD for arsenic via anchovy sauce intake was 2.63×10^{-7} mg/kg/day and LADD was 8.42×10^{-8} mg/kg/day. The non-carcinogenic effects from exposure to arsenic in term of HQ ranged from 6.0×10^{-6} to 4.9×10^{-3} with mean value 0.013. HQ less than 1 indicated that there was no risk of any adverse health effects. While, the LCR for As exposure by anchovy sauce intake ranged from 1×10^{-9} to 1.1×10^{-6} with mean value 2.16×10^{-7}. According to the HQ and LCR value, it showed the value was still in acceptable range. Table 11 summarizes the results of ADD, LADD, HQ, and LCR.

Mean value of arsenic concentrations in anchovy sauce samples was 2807 mg/g. An average amount of intake rate was 0.007 g/day. Exposure duration was selected from an average value of 26 years with ranged 18 to 59 years and the average of exposure frequency was 444 days/year. Mean body weight from the present data was 60 kg. Averaging time is fixed to 25,550 days for LADD estimation and ADD is equal to exposure duration multiplied by 365 days. These parameters characteristic were described in Table 10.

Table 10 Description of parameters used for estimating risk

Parameter	**Symbol**	**Units**	**Parameter characteristic**
Concentration of arsenic	C	mg/g	2807
Ingestion rate	IR	g/day	0.007
Exposure duration	ED	Years	26
Exposure frequency	EF	days/year	444
Body weight	BW	Kg	60
Averaging time (carcinogenic)	AT	Days	25,550
Averaging time (non-carcinogenic)	ATc	Days	9414
Reference dose	ATnc	mg/kg/day	0.0003
Cancer slope factor	RfD	(mg/kg/day)-1	1.5

Source: *(Saipan, 2009)*

Table 11 Results of health risk assessment

Results	Median (IQR)	Range
Non-carcinogenic effects		
ADD (mg/kg/day)	2.63×10^{-7} (3.5×10^{-7})	0.1×10^{-7} - 1.47×10^{-6}
HQ	8.77×10^{-4} (0.011)	6.0×10^{-6} - 4.9×10^{-3}
Carcinogenic effects		
LADD (mg/kg/day)	8.42×10^{-8} (1.6×10^{-7})	0.1×10^{-7} - 7.4×10^{-7}
LCR	1.26×10^{-7} (2.44×10^{-7})	1×10^{-9} – 1.1×10^{-6}

Table 12 Health risk of the residents

Health risk		N	%	Mean± S.D	Range
HQ					
<1	Acceptable	160	100	0.019 ± 0.074	9.47×10^{-7}-0.39
>1	Unacceptable	0	0	-	-
LCR					
$<10^{-6}$	Clearly Acceptable	160	100	$2.16 \times 10^{-7} \pm 2.22 \times 10^{-7}$	1×10^{-9}-1.4×10^{-6}
10^{-6}-10^{-4}	Acceptable	0	0	-	-
$>10^{-4}$	Unacceptable	0	0	-	-

4.6 Source of other arsenic exposure to human

Human can be exposed to As through drinking water, cigarette smoking, insecticide and pesticide usage as demonstrated in Table 13. From the questionnaire data, water sources in Kg Pengkalan Kubur consist of tap water and well water. The majority of the respondents in this village used well water for their daily usage which contributed to 83% of usage. Some of them used tap water and both tap water and well water. Only 5% from the respondents using pesticide in their life. While, others choose to not used pesticide (95%). Majority of the respondents did not smoke (74%) but most of the respondents were used insecticide (66%).

Table 13: Source of other arsenic exposure to human

Variables	Frequency	Percentage
Water sources		
Tap water	18	11
Well water	133	83
Well water and tap water	9	6
Smoking		
Yes	36	23
No	119	74
Quit smoking	5	3
Pesticide usage		
Yes	8	5
No	152	95
Insecticide usage		
Yes	106	66
No	54	34

* N=160

4.7 Frequency intake of anchovy sauce consumption and the lifetime cancer risk encountered by villagers

According to the health risk assessment, the results showed a significant relationship between the frequency intake of anchovy sauce consumption and the LCR encountered by villagers since (p<0.0001). As shown in the scatter plot graph, there was a positive correlation between the frequency intake of anchovy sauce consumption and the LCR encountered by villagers.

Table 14 Relationship between the frequency intake of anchovy sauce consumption and the lifetime cancer risk encountered by villagers.

Variable	Frequency of anchovy sauce consumption	
	r	**P**
Lifetime cancer risk	0.666	<0.0001

**. Correlation is significant at the 0.01 level (2-tailed).

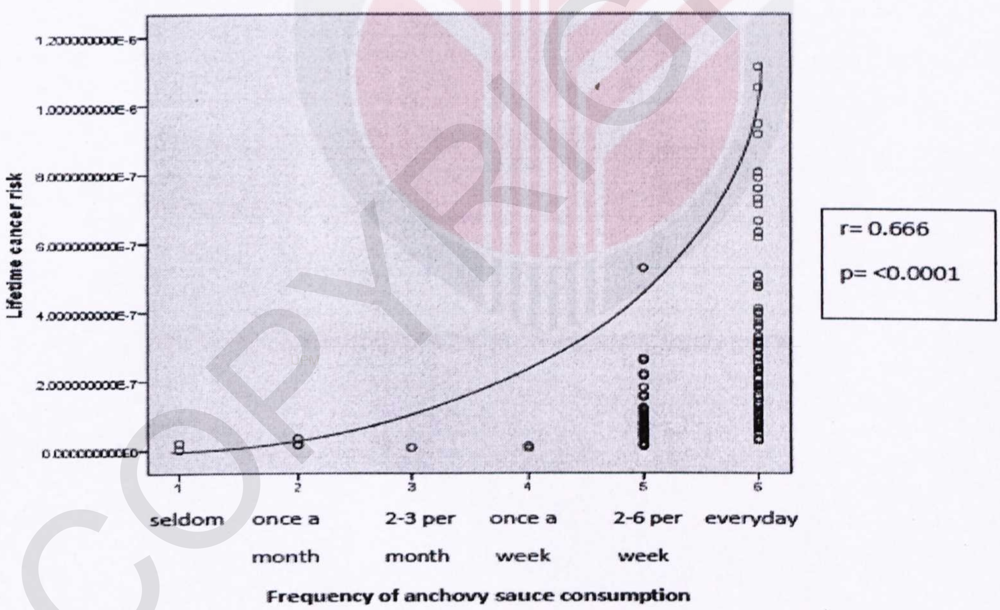

Figure 13 Relationship between frequency of anchovy sauce intake and lifetime cancer risk.

From the health risk assessment also, result showed a significant relationship between the frequency intake of anchovy sauce consumption and the HQ encountered by villagers since ($p<0.001$). Based on the scatter plot graph in Figure 9 there was a positive correlation between these two variables.

Table 15 Relationship between the frequency intake of anchovy sauce consumption and the hazard quotient encountered by villagers.

Variable	Frequency of anchovy sauce consumption	
	r	**P**
Hazard quotient	0.759	<0.0001

**. Correlation is significant at the 0.01 level (2-tailed).

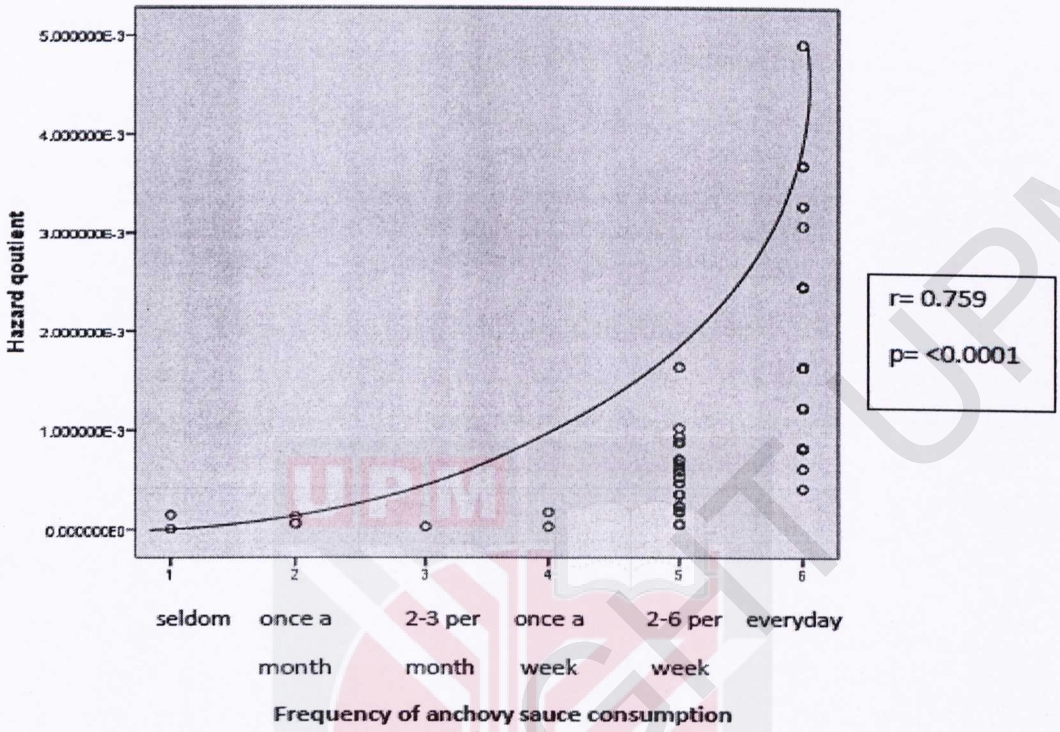

Figure 14 Relationship between frequency of anchovy sauce intake and hazard quotient

4.8 Association between frequency of anchovy sauce consumption and arsenic signs

Results show no significant.

Table 16 Association between the frequency intake of anchovy sauce and the prevalence of acute arsenic poisoning signs among villagers

Variables Acute symptoms		Frequency of anchovy sauce intake Frequent	Less frequent	X^2	p
Stomach ache	Yes	1	0	0.689	0.407
	No	94	65		
Vomit	Yes	0	0	0.00	0.00
	No	95	65		
Diarrhea	Yes	0	1	1.471	0.225
	No	95	64		
Muscle cramp	Yes	2	2	0.149	0.699
	No	93	63		
Headache	Yes	7	4	0.089	0.766
	No	88	61		
Difficulty breathing	Yes	1	0	0.689	0.407
	No	94	65		
Fatigue	Yes	4	5	0.881	0.348
	No	91	60		
Allergic	Yes	4	4	0.307	0.580
	No	91	61		

*N=160

Table 17 Association between the frequency intake of anchovy sauce and the prevalence of chronic arsenic poisoning signs among villagers

Variables Chronic symptoms		Frequency of anchovy sauce intake Frequent	Less frequent	X^2	p
Hyperkeratosis	Yes	0	0	0.00	0.00
	No	95	65		
Rashes	Yes	2	0	1.386	0.239
	No	93	65		
Hair loss	Yes	2	3	0.803	0.370
	No	93	62		
Brittle nail	Yes	0	1	1.471	0.225
	No	95	64		
Numb at hand	Yes	8	7	0.250	0.617
	No	95	65		
Cancer	Yes	2	1	0.067	0.795
	No	93	64		

*N=160

CHAPTER 5

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Discussion

5.1.1 Socio-demographic information of the respondents

The proportions of female in this study were higher than male because most of them were housewives. During the study was conducted, there were only housewives at home while the male was out to work. Age ranges from 18 to 29 years old were the highest population. Most of them among students who were having semester break of the university session.

From the questionnaire, the study found that most of the respondents were in healthy state as indicated by body mass index (BMI) scale. Some of them were overweight and obese which put them all risk in chronic diseases. From the previous study, obesity was not necessarily a problem affecting only the rich but also can affects among those whose household income was in the middle income group (Mariam *et al.*, 2009). Regarding to that study also, some underweight problem was caused by low knowledge in nutrition among respondents.

Majority of the respondents had low monthly income in which can be related with the types of occupation that they had. There were self-employed in particularly as fisherman. Basically, the income for a fisherman was not fixed. It is depends on the daily caught.

5.1.2 Food frequency

It was found that anchovy sauce was relatively favorable to the majority of the respondents as more than 50% of the respondents consumed anchovy sauce every day. It is because anchovy sauce is the traditional food and one of the best known fermented seafood products in Kelantan as well as Southern Thailand. One of the reasons for the increase in the consumption of fermented foods because traditionally; 'budu' was used as a food enhancer in countless Malaysian dishes.

Marine fish, freshwater fish, anchovy, canned fish and shrimp were the example of the food that possibly contributes to the detection of arsenic during this study. These foods were most consumed by the respondents. In a study that was conducted by

Naransal (2001) in Semenyih, anchovy was detected contained arsenic in amount of 0.023 ± 0.01 mg/kg. While, the average arsenic content in freshwater fish is of 0.54 lg/g total wet weight (Sharif *et al.*, 2008). It is been said that the marine organism accumulate more arsenic than fresh-water organisms. Likewise with the results by Yost (1998) study proved that marine species that were highly consumed by respondents tend to accumulate much higher levels of arsenic than freshwater species.

The study also confirmed that, rice has higher As concentrations than most other foods. Diets that rely heavily on these foods may be exposing to high As concentration. Finding from questionnaire showed that most of the respondents consumed these foods everyday. This result was expected as rice was the staple food of Malaysians. The other food items eaten daily but by a smaller proportion of the respondents such as freshwater fish, cabbage ,bean vegetable, anchovy, shrimp paste ,shrimp and canned fish. All these latter food items were consumed at least once a day and also contribute to arsenic intake.

Leafy vegetables were consumed in high proportion by respondents in this study. These foods also has been stated to be contaminated by As due to the agricultural activity.The use of pesticide might be contribute to the As contamination in soil. As hightlight by Helgesen & Larsen (1998) in their studies, that there was a potential movement of As species from soil into agronomics crops. The study reveal soil can be another source of arsenic exposure for agricultural crop. However, the agricultural activity in the study area was not the main activity. Respondents got the vegetables from the market supply which means the As contamination in vegetables might be from other area. As proven by Bernhard *et al* (2004) that studied on the heavy metals in soils and crops in Peninsular Malaysia. The finding from his study reveals that the addition of phosphatic fertilizers to the agricultural soils has resulted increasing in heavy metal such as As. The reason was majority of cultivated soils of Peninsular Malaysia are heavily fertilized with phosphatic fertilizers.

5.1.3 Arsenic concentration

The present study found that the value of As concentration in anchovy sauce sample compared to the values from Food Regulation 1985 showed it exceed the safe level consumption. As concentration that permitted by Food Regulation 1985 was not more than 1 mg/kg. In addition, the concentrations also exceeded the maximum standard limit of 0.015 mg/kg of PTWI for human consumption per week when multiplying with seven.

As proven by studies in the USA (Gunderson, 1995, Yost *et al.,* 1998) the highest concentrations of total As was found in seafood. The reason why As was high in seafood because the As exposure can be from various sources. As proved by Naransal

(2001), the concentration of As was detected mostly in seafood because sea was more exposed to the several of pollutants compared to other places.

This is supported by Juresa (2010) study who found that concentration As in seafood exceeded permitted level set by Croatian Ministry of Health. The estimated values of an average person's weekly intake of inorganic arsenic from examined species were in the range 0.02–0.85 mg, which represents 2–81% of the PTWI.

By contrast, finding from Thailand and Vietnam studies (Rodriguez, 2009), the total As concentrations of six different fish sauces were in the range of 0.69–2.75 mg/l. This value was below the detection limit and within the safe level compared to the present study.

5.1.4 Acute and chronic arsenic poisoning signs

Arsenic is toxic to humans. The common acute signs that experienced by the respondents such as headache, fatigue, allergic and muscle cramp. These acute signs might be due to the work hard and work in direct sunlight. This finding was similar with the previous study by Mandal (2002) that reported the acute sign of As include profound gastro-intestinal damage which are resulting in severe vomiting, diarrhea and muscular cramps.

Data from questionnaire showed that numbness was the most common chronic arsenic sign experienced by the respondents. Other symptoms like hair loss, rashes and brittle nail were reported low among respondents. This might be due to the occupation of respondents. Majority of them work as fisherman and had aged more than forty years and above. Hyperkeratosis was not reported for the chronic arsenic poisoning signs due to the small intake of anchovy sauce. Hyperkeratosis one of the sign for skin disorders that were documented in several epidemiological studies.

5.1.4 Source of other arsenic exposure to human

People were exposed to the As through drinking water, cigarette smoking, pesticide and insecticide usage. Drinking water was a potential source of total arsenic dietary exposures. Generally, arsenic in water is inorganic form and are the most toxic compared to seafood which is organic form. For crop production, the use of agricultural chemicals such as pesticides and fertilizer also contribute to the arsenic contamination.

Smoking was another possible source for arsenic exposure. According to the Air Quality Guidelines, people who smoke for about 20 cigarettes per day was estimated to ingest 0.7–2.1 (µg/day) daily intake of arsenic (Hughes, 1994). Only certain male respondents were smoke because culturally, women do not smoke in local community because it was considered negative behavior.

5.1.5 Relationship between frequency of anchovy sauce consumption and health risk encountered by villagers

There was a positive correlation between the frequency intake of anchovy sauce consumption and the LCR encountered by villagers. There were no respondents who exceed the acceptable limit value. It is mean all respondents are in safe level. This is might be due to the short duration of anchovy sauce intake since majority respondents at young age. If the amount of food intake is higher due to the continuously consumption, there was a possibility of the LCR will increase. Same goes to the relationship between the frequency intake of anchovy sauce consumption and the HQ encountered by villagers. There was a positive correlation between these two variables. It means that, the intake of anchovy sauce influence the HQ value. The frequency of anchovy sauce intake will cause the hazard quotient increase. As mention by WHO (2010), by intake of small quantities of organic arsenic via seafood does not pose a health risk.

5.1.6 Association between frequency of anchovy sauce consumption and arsenic signs

According to the results, the association between frequency of anchovy sauce consumption and the acute and chronic arsenic poisoning signs showed there was no significant association. All the signs too common and it is not significant for As poisoning sign. After all, data from questionnaire was not suitable in studying the As signs in low doses. The best method is to perform the biological monitoring.

5.1.7 Study limitation

One of the important reasons for higher concentration of arsenic in anchovy sauce sample was the formation of ArCl+ (m/z 75, the same nominal mass as As+) in the argon plasma of the ICPMS. So that, the sample was not well tolerated, and cause spectral interference chloride ions. Hence, gives high value of As detection. The best equipment for arsenic detection was by using Atomic Absorption Spectrophotometry (AAS).

5.2 Conclusion

Arsenic was detected in high level in sample of anchovy sauce which exceeds the standard level of safe consumption by Food Regulation 1985. More than 50 % of respondents consumed anchovy sauce in average 1 teaspoon daily. From the health risk assessment calculation, the LCR and HQ were still in acceptable level. There was a significant relationship between the frequency of anchovy sauce intake and the health risk encountered by villagers. However, there is no significant association between the frequency of anchovy sauce intake and acute and chronic arsenic poisoning signs. The health risk assessment showed HQ and LCR level not exceed 1 which indicate acceptable level which respondents might not manifested the health effect yet.

5.3 Recommendation

Community should aware the health effects due to high consumption of anchovy sauce continuously. Community were suggested to consume food that contain sulphur and fibers such as eggs, fruits and vegetables in order to detoxify the As in our body. This nutritional intake was useful and it actually was applied traditionally. Food that contains sulphur protects cells from the effects of toxins and assists in the formation of bile. Food that rich with fibers aids in detoxification since toxins will adhere to fibers and be eliminated as waste (Katelyn, 2012).

Regular inspection to this food should be performed from time to time in order to monitor the level of As concentration. The needs of a monitoring to ensure a safe food supply to the consumer. Cooperation with Ministry of Health should be done in order to have better impacts on monitoring process. Manufacturer also should improved food hygienic during food preparation to avoid the food products to be contaminated by heavy metals especially As.

Other than that, biological monitoring should be performing to evaluate the presence of As in past or long term exposure. Hair and nail samples are good examples for As biological monitoring (Hinwood.,*et al* 2003). This is because the stability of arsenic in hair and nail was expected to be high.

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APPENDIX 1

Flow chart of *budu* production

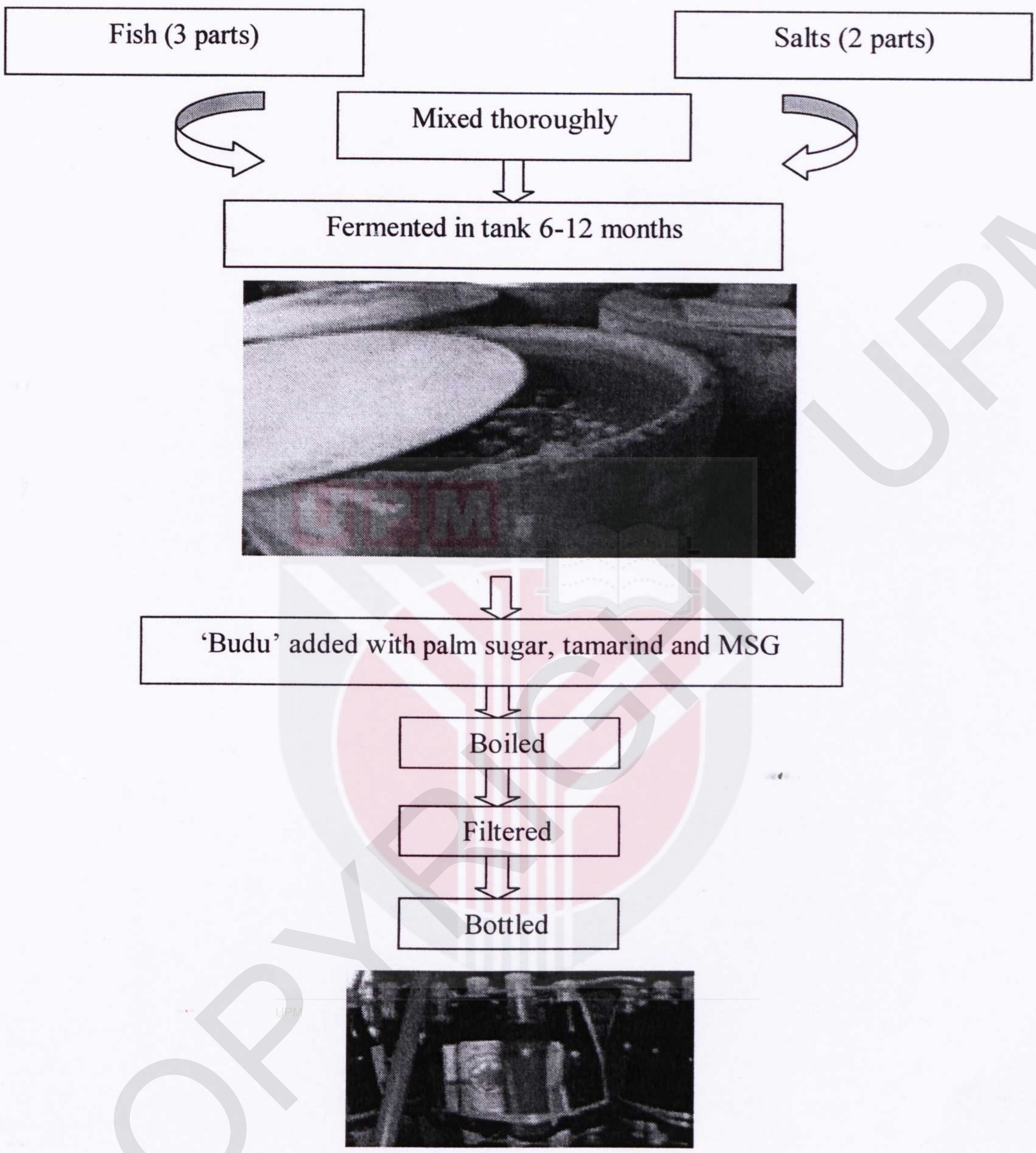


Figure 3 Flow chart of 'budu' production *(Source: Rosma, 2009)*

APPENDIX 2

Conceptual framework

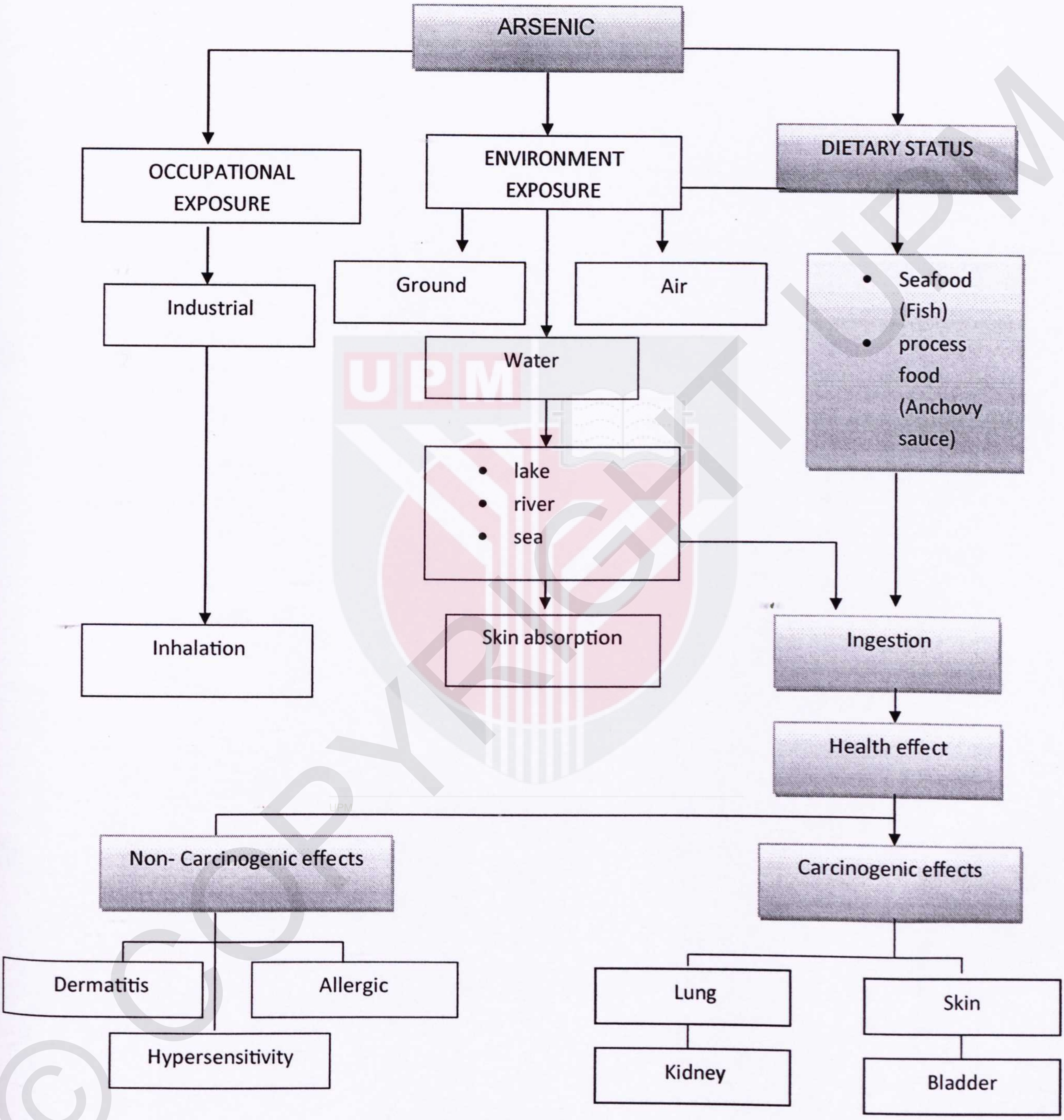


Figure 4: Conceptual framework chart

APPENDIX 3

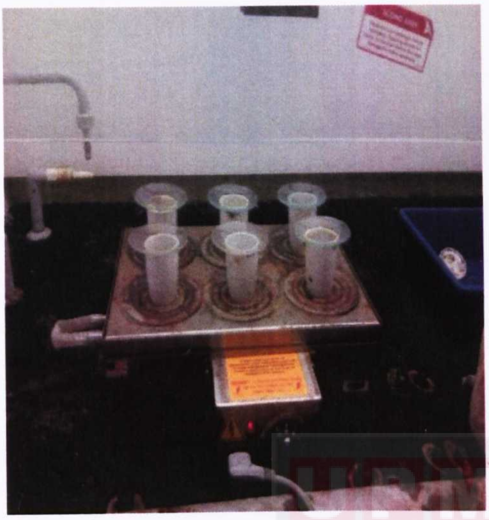
Gann chart

GANNT CHART –RESEARCH SCHEDULE TABLE

Table 18 Gannt chart table

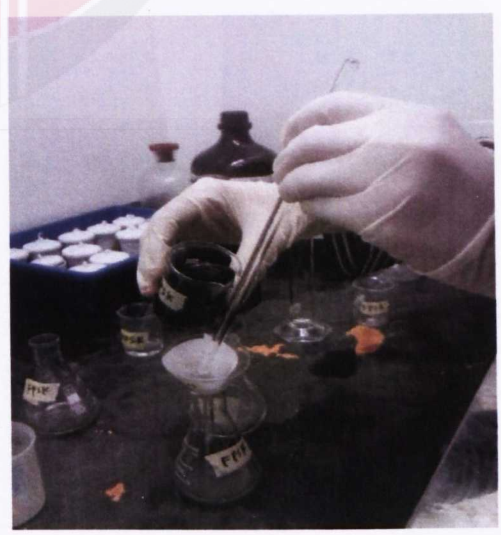
ACTIVITY	2011				2012				
	9	10	11	12	1	2	3	4	5
Research proposal									
Proposal approval									
Data collection									
Data analysis									
Report writing									

	
10 g of the anchovy sauce sample was weighted on analytical balance	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. After that, the sample was removed from the furnace and cooled down



20 ml of 5 M HNO_3 was added and evaporated to dryness on a water bath

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)



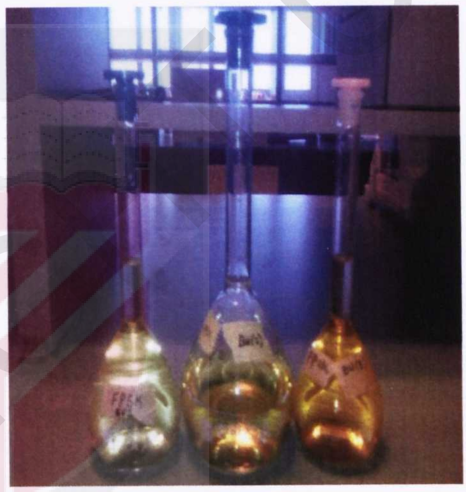
20 ml of concentrated HCl was added and the sample was evaporated to dryness, removed, and then again 50 ml of 2 M HCl was added and the tube was again swirled	Solution was filtered through Whatman No. 42 filter paper and <0.45 lm Millipore filter paper
 Solution was transferred quantitatively to a 250 ml volumetric flask by adding deionised water	 The final solution of the three samples

Figure 17 Dry ashing work flow

APPENDIX 4

Respondent Information Sheets

Respondent Informed Consent Form

Questionnaire

Study approval letter from head villagers

Medical Research Ethic Committee Approval Letter

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PENERANGAN KEPADA PESERTA

TAJUK KAJIAN: PENCEMARAN LOGAM BERAT ARSENIK DI DALAM SOS IKAN BILIS (BUDU) DAN PENILAIAN RISIKO KESIHATAN DI KALANGAN PENDUDUK DI TUMPAT,KELANTAN.

PENYELIDIK : SUZILLA BT YAACOB

Terima kasih kerana membantu kami di dalam kajian ini.

Apakah kajian ini?

Sejak kebelakangan ini,air semulajadi telah dicemari oleh bahan toksik daripada pelbagai sumber.Spesies hidupan laut kini semakin terancam bukan sahaja kerana kewujudan logam secara semulajadi di dalam laut tetapi juga hasil daripada aktiviti manusia.Spesies ikan terutamanya ikan bilis digunakan sebagai bahan utama dalam pemprosesan budu.Disebabkan ada pencemaran air dan kemungkinan ikan bilis yang digunakan dalam pemprosesan budu juga dicemari oleh logam arsenik.Masyarakat yang mengambil budu sebagai menu harian mereka mungkin akan dicemari oleh logam arsenik di dalam sistem badan mereka.

Apakah tujuan kajian ini?

Kajian ini dijalankan bertujuan untuk mengkaji pencemaran logam berat arsenik di dalam sos ikan bilis (budu) dan penilaian risiko kesihatan terhadap penduduk di Tumpat,Kelantan.

Berapa ramai responden yang terpilih?

Responden akan dipilih daripada kalangan penduduk yang tinggal berdekatan dengan kawasan pemprosesan sos ikan bilis (budu) di sekitar Tumpat .Seramai 158 orang responden dari Kg. Pengkalan Kubur 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 dan ketinggian akan diambil untuk mengetahui 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.

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Terima kasih atas kerjasama dan bantuan anda.

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BORANG PERSETUJUAN RESPONDEN

TAJUK KAJIAN: PENCEMARAN LOGAM BERAT ARSENIK DI DALAM SOS IKAN BILIS (BUDU) DAN PENILAIAN RISIKO KESIHATAN DI KALANGAN PENDUDUK DI TUMPAT,KELANTAN

PENYELIDIK : SUZILLA BT YAACOB

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 berkaitan

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)

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BORANG PERSETUJUAN IBUBAPA/GRADUAN

TAJUK KAJIAN: PENCEMARAN LOGAM BERAT ARSENIK DI DALAM SOS IKAN BILIS (BUDU) DAN PENILAIAN RISIKO KESIHATAN DI KALANGAN PENDUDUK DI TUMPAT,KELANTAN

PENYELIDIK : SUZILLA BT YAACOB

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 berkaitan

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 dikalangan penduduk di Kg. Pengkalan Kubur,Kelantan

Adalah dimaklumkan bahawa satu kajian tentang kesihatan akibat pendedahan kepada kepekatan arsenik dalam budu 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) | | | | - | | | | | | | | |

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BAHAGIAN A	KETERANGAN DIRI	RUANGAN KOD

Tandakan (√) dalam kotak berkenaan.

Nama Penuh: ……………………………………………………………………

Alamat Semasa: ……………………………………………………………………

……………………………………………………………………………………

1. Umur: ……………. Tahun

2. Jantina: ☐ Lelaki ☐ Perempuan

3. Bangsa: ☐ Melayu ☐ Cina ☐ India
   ☐ Lain-lain (Sila nyatakan): …………………………….....

4. Agama:
   ☐ Islam
   ☐ Kristian
   ☐ Buddha
   ☐ Hindu
   ☐ Lain-lain

5. Status:
   ☐ Bujang
   ☐ Berkahwin
   ☐ Bercerai/berpisah
   ☐ Balu/Duda

6. Taraf pendidikan:
   ☐ Tidak Bersekolah
   ☐ UPSR
   ☐ PMR
   ☐ SPM
   ☐ Sijil/STPM/Matrikulasi
   ☐ Diploma
   ☐ Ijazah Sarjana Muda
   ☐ Sarjana Muda
   ☐ Doktor Falsafah

7. Jenis pekerjaan:
   ☐ Kerajaan
   ☐ Swasta
   ☐ Bekerja Sendiri
   ☐ Pencen/Tidak Bekerja
   ☐ Lain-lain

ID PESERTA ☐☐☐

8. Pendapatan sebulan (RM) : _______________

9. Pendapatan isi rumah sebulan (RM) : ______________

10. Bilangan ahli isirumah : ___________ orang

BAHAGIAN B	MAKLUMAT KESIHATAN	RUANGAN KOD

1. Adakah anda mengalami masalah kesihatan berikut?

Simtptom 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-simptom diatas?

☐ Ya, nyatakan kali terakhir anda mendapatkan rawatan

____

☐ Tidak

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BAHAGIAN C:	ANTHROPOMETRI	RUANGAN KOD
1. Berat (kg) :	1. i. ☐☐☐ . ☐ kg	
2. Tinggi (cm) :	2. i. ☐☐☐ . ☐ cm	

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

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

Klasifikasi ⇨

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

ID PESERTA: ☐☐☐

BAHAGIAN D: BORANG KEKERAPAN PENGAMBILAN MAKANAN

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

Kod	Jenis makanan	Kekerapan pengambilan					Ukuran sajian(pilih satu jenis ukuran sahaja)	Berapa banyak sajian setiap kali makan
	(B)Ikan dan makanan laut	Berapa kali sehari	Berapa kali seminggu	Berapa kali sebulan	Berapa kali setahun	Tidak makan		
B1	Ikan laut						Keping	
							Ekor	
B2	Ikan air tawar						Keping	
							Ekor	
B3	Ikan bilis						Sudu makan	
B4	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	Kekerapan pengambilan					Ukuran sajian(pilih satu jenis ukuran sahaja)	Berapa banyak sajian setiap kali makan
	(C)Kekacang dan hasilnya	Berapa kali sehari	Berapa kali seminggu	Berapa kali sebulan	Berapa kali setahun	Tidak makan		
C1	Kekacang						Sudu makan	
C2	Tauhu						Keping	
C3	Kacang Tanah						Sudu makan	

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

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

Kod	Jenis makanan (E)Makanan perencah /perasa	Kekerapan pengambilan Berapa kali sehari	Berapa kali seminggu	Berapa kali sebulan	Berapa kali setahun	Tidak makan	Ukuran sajian(pilih satu jenis ukuran sahaja)	Berapa banyak sajian setiap kali 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 Berapa kali sehari	Berapa kali seminggu	Berapa kali sebulan	Berapa kali setahun	Tidak makan	Ukuran sajian(pilih satu jenis ukuran sahaja)	Berapa banyak sajian setiap kali 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 : ______________________

ID PESERTA: ☐☐☐

BAHAGIAN E:	FAKTOR-FAKTOR PENDEDAHAN LAIN	RUANGAN KOD

Tandakan (√) dalam kotak berkenaan.

Sumber bekalan air

1. Dari manakah anda mendapat sumber bekalan air minuman?

☐ Air paip

☐ Air perigi

☐ Lain-lain :______________

Pendedahan pekerjaan

2. Pernahkah pekerjaan anda melibatkan penggunaan pestisid ?

☐ Ya

☐ Tidak

Jika Ya, sila ke soalan seterusnya. (3) jika Tidak, sila terus ke soalan (4)

3. Berapa lama anda terlibat dengan pekerjaan ini?

____

4. Adakah anda menggunakan racun serangga selain daripada waktu bekerja?

☐ Ya

☐ Tidak

Amalan gaya hidup

a) Merokok

5. Adakah anda merokok?

☐ Ya

☐ Tidak

☐ Sudah berhenti

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

Bil.tahun: ____________ Bil. Batang rokok sehari ________

6. Semasa anda merokok,adakah anda menyedut asap rokok?

☐ Tidak sama sekali

☐ Sedikit

☐ Sederhana

☐ Mendalam

Jika sudah berhenti, sila jawab soalan 7

7. Jika sudah berhenti,pada umur berapa anda berhenti merokok sepenuhnya?

____

b) Pengambilan alkohol

8. Adakah anda pernah mengambil minuman beralkohol?

☐ Ya

☐ Tidak

Jika ya, sila nyatakan berapa botol sehari anda minum?

__________________ botol

APPENDIX 5

ICP-MS's result sheets

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					ppb
Cd	111					ppb
Pb	208					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

Quantitative Analysis - Summary Report

Sample ID: 11 (16)

Sample Date/Time: Wednesday, March 28, 2012 12:35:46
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)\11.021
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	156289	2.527	216.003	7.911
Cd	111	2412	3.267	113.334	14.264
Pb	208	53793	1.561	200.002	10.149

Concentration Results

Analyte	Mass	Net Intens. Mean	Conc. Mean	Conc. SD	Conc. RSD	Sample Unit
As	75	156072.541	**119.513**	3.02	2.5	ppb
Cd	111	2298.986	**1.321**	0.05	3.4	ppb
Pb	208	53593.376	**3.183**	0.05	1.6	ppb

Quantitative Analysis - Summary Report

Sample ID: 12

Sample Date/Time: Wednesday, March 28, 2012 12:37:30
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)\12.022
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	155972	1.994	216.003	7.911
Cd	111	2402	0.855	113.334	14.264
Pb	208	52799	5.616	200.002	10.149

Concentration Results

Analyte	Mass	Net Intens. Mean	**Conc. Mean**	Conc. SD	Conc. RSD	Sample Unit
As	75	155756.301	**119.271**	2.38	2.0	ppb
Cd	111	2288.316	**1.315**	0.01	0.9	ppb
Pb	208	52598.530	**3.124**	0.18	5.6	ppb

Quantitative Analysis - Summary Report

Sample ID: 13

Sample Date/Time: Wednesday, March 28, 2012 12:39:13
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)\13.023
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	154728	1.070	216.003	7.911
Cd	111	3509	4.264	113.334	14.264
Pb	208	37189	4.554	200.002	10.149

Concentration Results

Analyte	Mass	Net Intens. Mean	**Conc. Mean**	Conc. SD	Conc. RSD	Sample Unit
As	75	154512.399	**118.319**	1.27	1.1	ppb
Cd	111	3396.011	**1.952**	0.09	4.4	ppb
Pb	208	36989.348	**2.197**	0.10	4.6	ppb

Quantitative Analysis - Summary Report

Sample ID: 14

Sample Date/Time: Wednesday, March 28, 2012 12:40:58
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)\14.024
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	156723	0.242	216.003	7.911
Cd	111	3489	6.570	113.334	14.264
Pb	208	37036	4.167	200.002	10.149

Concentration Results

Analyte	Mass	Net Intens. Mean	**Conc. Mean**	Conc. SD	Conc. RSD	Sample Unit
As	75	156506.701	**119.846**	0.29	0.2	ppb
Cd	111	3375.337	**1.940**	0.13	6.8	ppb
Pb	208	36836.040	**2.188**	0.09	4.2	ppb

Quantitative Analysis - Summary Report

Sample ID: 15

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

Sample File: C:\Elandata_LC-ICPMS\Sample\Nor Aisyah (FPSK).sam
Method File: C:\Elandata_LC-ICPMS\Method\nor aisyah (fpsk).mth
Dataset File: C:\Elandata_LC-ICPMS\DataSet\Nor Aisyah (FPSK)\15.025
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	147485	1.814	216.003	7.911
Cd	111	3168	1.355	113.334	14.264
Pb	208	34888	4.209	200.002	10.149

Concentration Results

Analyte	Mass	Net Intens. Mean	Conc. Mean	Conc. SD	Conc. RSD	Sample Unit
As	75	147268.977	**112.772**	2.05	1.8	ppb
Cd	111	3054.551	**1.755**	0.02	1.4	ppb
Pb	208	34688.227	**2.060**	0.09	4.2	ppb

Quantitative Analysis - Summary Report

Sample ID: 16

Sample Date/Time: Wednesday, March 28, 2012 12:44:26
Sample Description: Food samples
Solution Type: Sample
Blank File:
Number of Replicates: 3
Peak Processing Mode: Average
Signal Profile Processing Mode: Average
Dual Detector Mode: Dual
Dead Time (ns): 55

Sample File: C:\Elandata_LC-ICPMS\Sample\Nor Aisyah (FPSK).sam
Method File: C:\Elandata_LC-ICPMS\Method\nor aisyah (fpsk).mth
Dataset File: C:\Elandata_LC-ICPMS\DataSet\Nor Aisyah (FPSK)\16.026
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	146485	1.702	216.003	7.911
Cd	111	3248	1.623	113.334	14.264
Pb	208	37283	3.671	200.002	10.149

Concentration Results

Analyte	Mass	Net Intens. Mean	**Conc. Mean**	Conc. SD	Conc. RSD	Sample Unit
As	75	146268.969	**112.006**	1.91	1.7	ppb
Cd	111	3134.579	**1.801**	0.03	1.7	ppb
Pb	208	37083.028	**2.203**	0.08	3.7	ppb