UNIVERSITI PUTRA MALAYSIA

ARSENIC CONCENTRATION IN FRESHWATER FISH AND HEALTH RISK ASSESSMENT AMONG ADULTS IN TWO AREAS AT KLUANG, JOHOR

CHRISTOPHER GEORGE

ARSENIC CONCENTRATION IN FRESHWATER FISH AND HEALTH RISK ASSESSMENT AMONG ADULTS IN TWO AREAS AT KLUANG, JOHOR

BY

CHRISTOPHER GEORGE

Thesis submitted in fulfillment of the requirement for the degree of Bachelor Science (Environmental and Occupational Health) from the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia

ACKNOWLEDGEMENT

First of all, praise the Lord for all His blessings and guidance for this research project that can be completed. I offer my sincerest gratitude to my supervisor, Dr Saliza binti Mohd Elias and my co-supervisor, Dr Ahmad Zaharin Aris who had supported, advised and guided me throughout my research project and my thesis paper. I am really grateful and honoured being able to learn so many things from both of them which helped me to complete my research work.

This acknowledgement is also for the staff at FPSK Environmental Health Laboratory especially to Puan Norijah and Encik Mohd Nor for their help during the laboratory analysis of the research and also to all FPAS Laboratory staff in helping me to conduct the sample analysis at their laboratory. I would like to express my gratitude also to certain agencies that help to provide informations to me throughout my research project which are Jabatan Kemajuan Orang Asli (JAKOA) Kluang and Fishery Department of Kluang. I would also like to thank the village of Chiefs Kampung Sedohok and Kampung Punjut for their cooperation and also all the residents of the two villages that involved in this study.

Last but not least, I would like to thank my parents who had been supported me since this project started and not forgotten to my coursemates especially my Final Year Project groups and to my friends of Bachelor Science (Environmental and Occupational Health) for their help, cooperation and discussion during my research project.

ABSTRACT

ARSENIC CONCENTRATION IN FRESHWATER FISH AND HEALTH RISK ASSESSMENT AMONG ADULTS IN TWO AREAS AT KLUANG, JOHOR

CHRISTOPHER GEORGE

Introduction: Heavy metal contamination in food sources are more concern among the communities because of the effects to the human health. Arsenic can be found naturally in the environment and cannot be destroyed or degraded. The occurrences of arsenic to the environment are from the rapid industrialization, agricultural and uncontrolled urbanization which directly pollute the river basin that will contaminate the aquatic organism. Aquatic organism especially fish is one of the major part of the human diet due to the high protein source content. **Objective:** A cross-sectional comparative study was conducted at Kampung Sedohok and Kampung Punjut in Kluang, Johor in order to determine the arsenic concentration in freshwater fish of polluted river of Sembrong and less polluted river of Kahang and health risk assessment among adults in the two areas. This study also aimed to determine the socio demographic information and the fish frequency intake among respondents of both areas. **Methodology:** A total of 30 respondents from each Kampung Sedohok and Kampung Punjut were randomly selected based on the inclusion criteria. A set of pre-tested questionnaire was used to obtain the socio demographic information, food frequency intake and health status of respondents. Three species (N=6) of freshwater fish were caught from the selected rivers by the local fisherman using net method which later analyzed for arsenic concentration using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The health risk of respondents was indicated through the calculation of ADD, LADD, HQ and LCR. **Results:** All fresh water fish samples were detected with arsenic which range from 0.227 to 1.702 mg/kg and mean concentration of arsenic in freshwater fish for both areas do not exceeded the maximum concentration level stated in Malaysian Food Regulation 1985 (1 mg/kg). There was no significant difference between the arsenic concentrations in fresh water fish of both areas. However, there was a significant difference in the health risk of the respondents of the two villages. The results also showed that there was a significant relationship between the fish frequency intake with health risk of both HQ and LCR of the respondents in Kampung Sedohok. **Conclusion**: Arsenic concentration in fresh water fish in polluted river of Sembrong showed slightly higher as compared with less polluted river of Kahang. Biological monitoring should be assessed for future improvement of the study in order to determine the actual burden of arsenic in human body of the respondents.

Keywords: Arsenic, Fresh water Fish, ICP-MS, HQ, LCR, Health Risk Assessment

ABSTRAK

KEPEKATAN ARSENIK DALAM IKAN AIR TAWAR DAN PENILAIAN RISIKO KESIHATAN DI KALANGAN ORANG DEWASA DI DUA KAWASAN DI KLUANG, JOHOR

CHRISTOPHER GEORGE

Pengenalan: Pencemaran logam berat kepada sumber makanan menjadi perhatian dan keutamaan oleh masyarakat masa kini kerana kesannya kepada sistem tubuh yang boleh menjejaskan kesihatan seseorang individu. Arsenik merupakan unsur semulajadi dalam kerak bumi dan tidak boleh dimusnahkan. Pencemaran arsenic (As) berlaku melalui beberapa sumber seperti industri perkilangan dan penggunaan baja kimia dalam pertanian. Sumber-sumber ini mencemarkan air sungai dan secara langsung akan mendedahkan pencemaran tersebut kepada sumber protein iaitu ikan. **Objektif:** Satu kajian telah dijalankan di dua lokasi iaitu Kampung Sedohok dan Kampung Punjut di Kluang, Johor untuk mengenalpasti kepekatan arsenik dalam ikan air tawar di sungai tercemar, Sg.Sembrong dan sungai kurang tercemar, Sg. Kahang serta penilaian risiko kesihatan orang dewasa di dua kawasan tersebut. Objektif lain adalah untuk mengenalpasti maklumat sosio demografi penduduk dan kekerapan pengambilan ikan air tawar di dua kawasan tersebut. **Metodologi:** Seramai 30 orang responden dari setiap kampung telah dipilih secara rawak berdasarkan beberapa kriteria tertentu. Satu set borang soal selidik telah digunakan untuk menyelidik maklumat sosio demografi, kekerapan pengambilan makanan dan status kesihatan responden. Tiga spesies ikan air tawar (N=6) telah ditangkap daripada sungai-sungai yang dipilih oleh nelayan tempatan menggunakan jala ikan dan kemudiannya dianalisis menggunakan *Inductively Coupled Plasma Mass Spectrometry* (ICP-MS). Penilaian risiko kesihatan ditentukan dengan kiraan ADD, LADD, HQ dan LCR. **Hasil kajian:** Semua sampel ikan air tawar dikesan mengandungi As antara 0.227 hingga 1.702 mg/kg dan purata kepekatan As bagi setiap kampung adalah tidak melebihi tahap maksimum yang dibenarkan di dalam makanan oleh Peraturan- Peraturan Makanan 1985 iaitu 1 mg/kg. Hasil kajian mendapati tidak terdapat perbezaan yang signifikan kepekatan As dalam ikan air tawar di dua kawasan tersebut. Tetapi, terdapat perbezaan yang signifikan terhadap risiko kesihatan oleh penduduk bag kedua-dua kawasan. Keputusan kajian menunjukkan terdapat hubungan di antara kekerapan pengambilan ikan air tawar dan risiko kesihatan (HQ dan LCR) bagi Kampung Sedohok tetapi tidak pada Kampung Punjut. **Kesimpulan:** As dikesan dalam semua sampel ikan air tawar dengan purata kepekatan As yang tidak melebihi paras dibenarkan. Pemantauan biologi dicadangkan dijalankan kepada semua responden untuk menentukan tahap pendedahan sebenar terhadap As.

Kata Kunci: Arsenik, Ikan air tawar, HQ, LCR, Penilaian risiko kesihatan

TABLE OF CONTENTS

CHAPTER 3: METHODOLOGY

CHAPTER 4: RESULTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

As	Arsenic
ADD	Average Daily Dose
ATSDR	Agency for Toxic Substances and Disease Registry
BMI	Body Mass Index
DOE	Department of Environment
μg/L	Microgram per liters
HQ	Hazard Quotient
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
IRIS	Integrated Risk Information System
KP	Kampung Punjut
KS	Kampung Sedohok
LADD	Lifetime Average Daily Dose
LCR	Lifetime Cancer Risk
RFD	Reference Dose
USEPA	United State Environmental Protection Agency

CHAPTER 1

INTRODUCTION

1.1 Background

Nowadays, issues related to the heavy metal contamination in food sources are major concern especially among the communities because of the effects to the human health. Heavy metal can be found naturally in the environment and cannot be destroyed or degraded (ATSDR., 2007). Many studies and researches are being carried out related to the heavy metal contamination to the food. Heavy metals such as arsenic, cadmium, lead and chromium can contaminated the food sources through air, soil and through water pollution (Zukowska and Bizuk., 2008). The occurrences of all these heavy metals to the environment are from the rapid industrialization, agricultural and uncontrolled urbanization. All these activities can disperse the heavy metals to the environment and indirectly it can cause to the serious problems especially to the human health affected through the ingestion of the contaminated food source containing the elements of the heavy metal.

Arsenic is a naturally occurring element which widely distributed in the earth's crust. Arsenic combined with other elements such as oxygen, chlorine, and sulphur to form inorganic arsenic compounds. In animals and plants, it combines with carbon and hydrogen to form organic arsenic compounds (ATSDR., 2007). In the environment, arsenic can be found naturally in the soil and minerals and it may also enter the air, water and land. The characteristic of the arsenic like other metals is that, it cannot be destroyed in the environment; instead it can only change its form. The arsenic can also be released to the environment through the anthropogenic activities such as mining and smelting. Some studies showed that the sources of the arsenic pollution are from the emission and waste water of the ore mining and processing industry, dye manufacture facilities, tanneries, thermal power plants, and application of certain chemical used in agricultural activities (Sarkar and Datta., 2004). Other than that, there was also a study that showed through the anthropogenic sources, the aquatic environment is contaminated with arsenic through the activities such as agricultural, burning coal and operations of gold mining. (Horacio *et al.*, 2006). Shaw *et al.,* (2007) discussed that the arsenic is known to cause to adverse effects in the aquatic organisms and can cause the major problems of the human health by the intake of the contaminated aquatic organisms.

Aquatic organism such as fish is one of the main sources of food that rich source of vitamins, minerals and proteins. It is the richest source of an essentially healthy diet. Several studies show that the importance of fishes and their healthy benefits. They claim that fishes are the most healthy food with high source of omega 3 fatty acids, that brings a lot of benefits to human health, including the reduction of heart-related diseases (Castro-Gonzalez and Mendez-Armenta., 2008;Hajeb *et al*., 2009). In Malaysia, studies from Zuraini *et al*., (2006) and Tukiman *et al*., (2006) revealed that 60% to 70% of protein needs are fulfilled by the consumption of fishes. However, the fishes are endangered by contaminants transferred along the food chain. Among contaminants, heavy metals such as arsenic have been recognized as strong biological poisons because of their persistent nature and cumulative action (Carbonell-Barrachina *et al*., 2009). Arsenic has the tendency to accumulate in the bottom sediments. Essentially, fish has the characteristic to metabolize, concentrate and store water borne pollutants (EFSA., 2009). Indirectly, human exposed to the arsenic through the ingestion of the food such as the contaminated fish and also drinking water.

Exposure of arsenic can pose various health effects, acute effects such as irritation, allergic, skin disease (dermatitis) and lung irritation. Some studies show that arsenic can cause carcinogenic health effects to human health which causing the chronic effects such as lung, liver, skin and bladder cancer (Bernstam and Nriagu., 2000; Kapaj *et al*., 2006). Long term exposure of both organic and inorganic arsenic can pose serious problems in many organs such as disturbance of liver function, heart problems and chronic effects on the peripheral of nervous system.

1.2 Problem Statement

Contamination of fresh water rivers with a wide range of pollutants especially heavy metal contamination has become a serious problem. According to the Department of Environment (2010), there are more than 100 river systems in the Peninsular Malaysia and more than 50 river systems in Sabah and Sarawak. However, many of the rivers have become polluted due to the many effluents that have been discharges into the rivers. Several studies were done relating to the heavy metal pollution to the rivers in Malaysia. Kamaruzzaman *et al.,* (2008) showed that the Muar River in Johor had been heavily impacted by the discharges from municipal and industrial outflows. This was due to the rapid development of the area via the expansion of the industrialization area. The municipal and industrial discharges can be the sources of the heavy metal pollution to the rivers. According to the Department of Environment (2010), a total of 570 rivers were monitored for the water quality monitoring in Malaysia. The data showed that 293 rivers were found to be clean, 203 for slightly polluted rivers and 74 for polluted rivers. The comparison to the previous year shows the number of clean river and slightly polluted river decrease while the numbers of polluted rivers increase. These clearly showed that the fresh water rivers in Malaysia are endangered for heavy metal pollution especially to the living aquatic organisms such as fish.

There are several water pollution point sources that increase the polluted river with effluents such as heavy metal which comprise of manufacturing industries, sewage treatment plants, agro-based industry and animal farm (**Figure 1.1**). Other than that, statistic reported from Department of Environment (DOE) also showed that the state of Johor has the highest number of water pollution through the industrial water pollution sources which are from the agro-based and manufacturing industries (DOE., 2010).

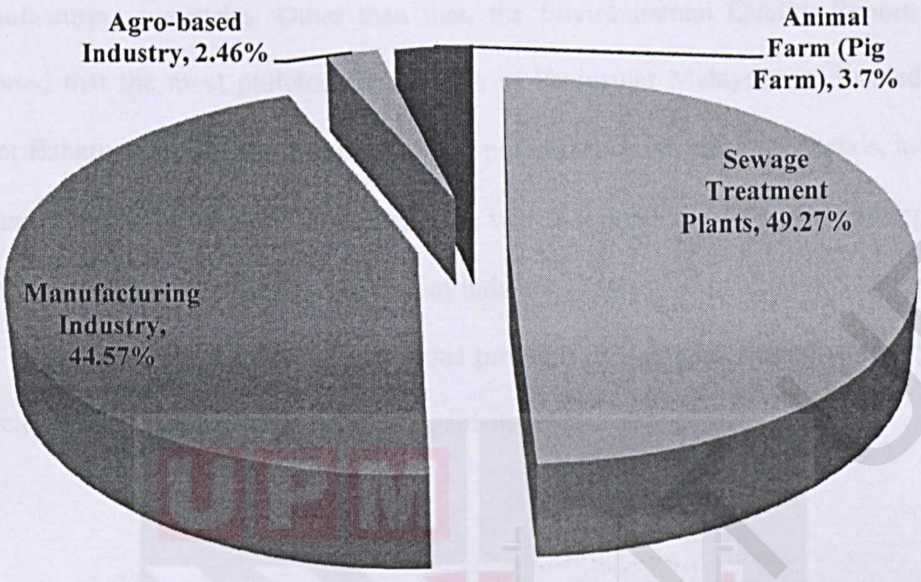

Figure 1.1: Composition of Water pollution sources by sector

(Source: Environmental Quality Report 2010, Department of Environment)

Johor is the third largest and one of the most developed states in Peninsular Malaysia. It is located in the southern of the Peninsular Malaysia. Rapid industrialization and uncontrolled urbanization causes the water pollution such as heavy metal contamination to the water resources especially the river basins in Johor. **Figure 1.2** shows that the distribution of industrial water pollution sources which in agro-based and manufacturing industries by state in Malaysia. It shows that Johor is the highest in the chart of the distribution industrial water pollution sources especially in agro-based and manufacturing industries. Other than that, the Environmental Quality Report (2008) reported that the most polluted river basins in Peninsular Malaysia are located in the Johor Baharu area. The main pollutants that pollute the Johor strait are metals, industrial organic chemicals, nutrients and pesticides which sources are from the anthropogenic activities of the agricultural and chemical industries (Bayen *et al.*, 2003). These showed that the river basin in Johor has a serious problem of pollution related to heavy metal which directly can effects the aquatic organisms.

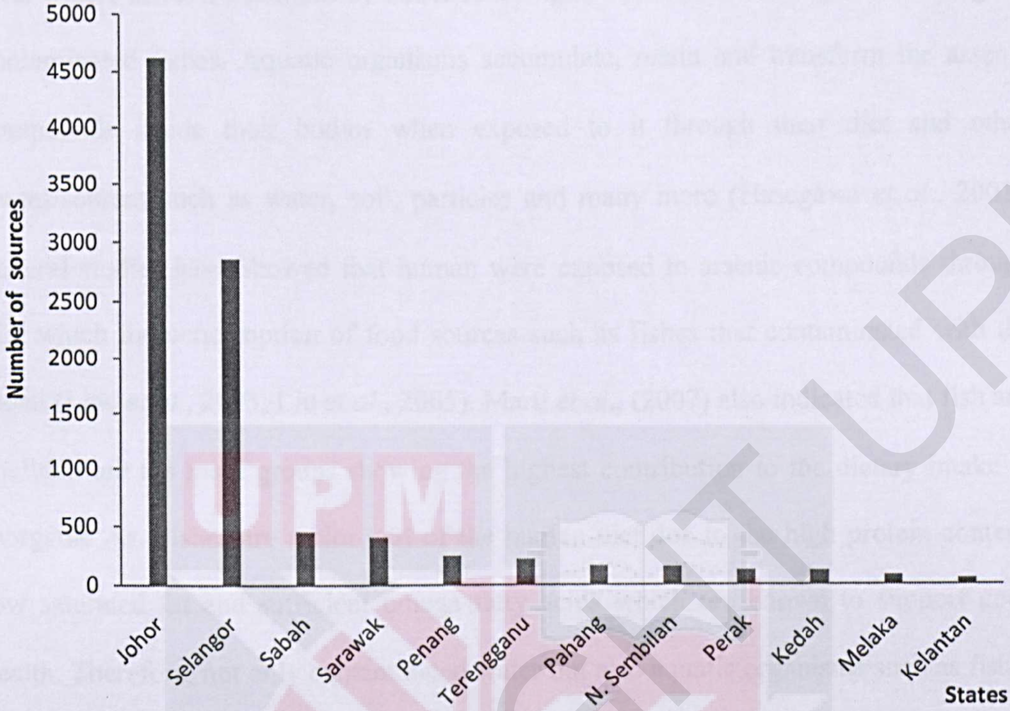

Figure 1.2: Distribution of Industrial water pollution sources (Agro-based and Manufacturing Industries) by state in Malaysia

(Source: Environmental Quality Report 2010, Department of Environment)

Heavy metal such arsenic can cause detrimental effects to the human health if exposed. Arsenic can be present in the soil, water, air, plants and other living organisms. In water, common forms of arsenic are As (III), As (V), methanearsonic acid and dimethyl-arsinic acid (EPA., 2000). According to Malik., (2004), metals accumulated in river waters infect the humans by direct consumption of water or through consuming the contaminated fishes. Aquatic organisms accumulate, retain and transform the arsenic compounds inside their bodies when exposed to it through their diet and other routes/sources such as water, soil, particles and many more (Hasegawa *et al*., 2001). Several studies have showed that human were exposed to arsenic compounds through diet which the consumption of food sources such as fishes that contaminated with the metal (Liao *et al*., 2003; Liu *et al*., 2005). Martí *et al*., (2007) also indicated that fish and shellfish are the main groups showing the highest contribution to the dietary intake of inorganic As. Fishes are major part of the human diet due to the high protein content, low saturated fat and sufficient omega fatty acids which are known to support good health. Therefore, not only contaminated water but also aquatic organisms such as fishes contaminated with arsenic may be potential sources to human health risks. Exposure arsenic can pose to serious health effects such as liver, lung and bladder cancer (Kapaj *et al*., 2006).

1.2 Study Justification

Arsenic exposure to aquatic environment need to be prevent because it can cause health problems for those people who consume it. According to the 14th Schedule of Malaysia Food Regulation 1985, the permitted arsenic level in the food sample must not exceed 1 mg/kg.

This study concern more about the level of heavy metal such as arsenic in the freshwater organism because it can effects the human health through the consumption of the contaminated fish and other aquatic organisms. Fish is selected as the sample because of the fish is primarily source of protein, vitamins, minerals and polyunsaturated fatty acids. It is also at the same time contains a reasonably large amount of heavy metals. Arsenic in the form of organic or inorganic is present naturally in the environment. It has the tendency to accumulate in bottom sediments (Smedley and Kinniburgh., 2002). Fish will be contaminated to the arsenic metal by ingestion of the particulate material suspended in water then the contaminated fish will be along the food chain and ingested by human. Fishes have been widely used as bio-indicators of pollution by metals. Muscle tissue of fish is the frequently used for analysis because of its major target tissue for metal storage and it is also the main edible part of the fish (Bhupander *et al.*, 2011).These shows that fish are excellent subject which can determine the contaminants present in the water samples. Studies related to heavy metals in fishes are important to ensure the food safety and protect human health. This is because heavy metals such as arsenic that polluted or discharges into the fresh water rivers or lakes can accumulate with the fresh water food through the food chain. Then, the intake of this food especially the contaminated fish can be harmful to human health. Han *et al*., (1998) reported that the consumption of contaminated fish and shellfish has been an important route of human exposure to trace elements. Other than that, arsenic exposure has been related to the appearance of some types of cancer such as lung, liver and bladder cancer.

In this study, two areas with different condition of environments were selected which were located in Kluang, Johor. Kluang was chosen for the study location because it is the centre of the freshwater river in Malaysia. The two areas that been selected were Kampung Sedohok and Kampung Punjut. Both of these villages were chosen based on the location which nearest to the river and the food sources especially fish consumption was directly from the selected location of the river. Kampung Sedohok was located near with the Sembrong River and the villagers took the fish for consumption from this river. There were many intensive industrial activities with different types of factories and agricultural activities of the river. Due to this, the Sembrong River was polluted with pollutant release by the near of industries. According to the Environmental Quality Report (2010) from Department of Environment (DOE) reported that Sembrong River as one of slightly polluted river which classified in Class III. Meanwhile, Kampung Punjut was located near at Kahang River which also source of fish consumption for the villagers. Based on the information given, there are no industrial activities located at the downstream region and less polluted. According to the Environmental Quality Report (2010) from Department of Environment (DOE) reported that Kahang River as one of clean river which classified in Class II.

Furthermore, most of previous studies related to heavy metal only focus on the determination of heavy metal concentration in food whereas there is less focus on health risks assessment in human. Therefore, the aim of this study is to determine the arsenic concentration in fresh water fish and to assess the health risk faced by the respondents who consume the contaminated fish.

1.4 Conceptual Framework

Figure 1.3 shows that the conceptual framework of the arsenic exposure which can expose to human health. Basically, human can expose to heavy metal such as arsenic in various ways. It can be through 3 major ways which are dietary intake, environmental sources and ccupational field. In the environment, it can be from man-made sources and the natural sources. Mining and smelting are some examples of arsenic exposure of man-made sources while for natural sources are from rock and volcanoes. Besides, human also can be exposed to the arsenic through the occupational field. Industrial process such as the process of mining and smelting can be the sources of the arsenic exposure which to human health especially the miners and the smelters. A person can expose to arsenic through dietary intake in a way of consumption of contaminated food with arsenic. The dietary intake can be divided into two categories which are animal sources and non-animal sources. The dietary intake can be through the consumption of contaminated of food such as contaminated fish. In this study, the contaminated fish is from the fresh water river. Next, the route of exposure to arsenic can either through several pathways for example through ingestion of contaminated fish. This can make human prone to get acute and chronic health effects. Acute health effects can cause non-carcinogenic risk while chronic health effects lead to carcinogenic risk.

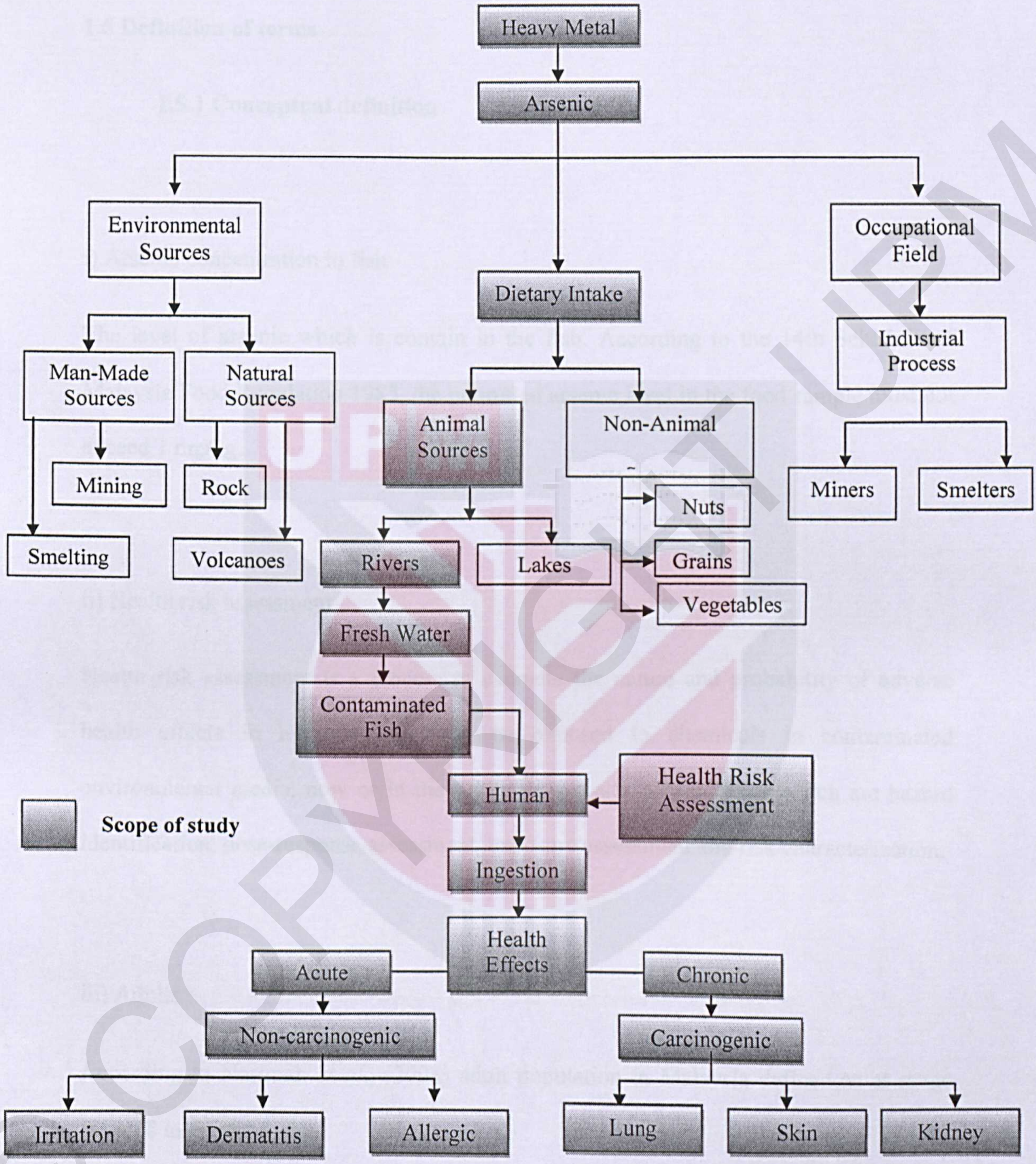

Figure 1: Conceptual framework of the route of arsenic contamination from aquatic environment to human

1.5 Definition of terms

1.5.1 Conceptual definition

i) Arsenic concentration in fish

The level of arsenic which is contain in the fish. According to the 14th Schedule of Malaysia Food Regulation 1985, the permitted arsenic level in the food sample must not exceed 1 mg/kg.

ii) Health risk assessment

Health risk assessment is a process to estimate the nature and probability of adverse health effects in humans who may be exposed to chemicals in contaminated environmental media, now or in the future. It includes 4 basic steps which are hazard identification, dose-response assessment, exposure assessment and risk characterization.

iii) Adults

According to Norimah *et al*., (2008) adult population in Malaysia defined as at range from 18 to 59 years old.

1.5.2 Operational definition

i) Arsenic concentration in fish

In this study, the concentration of arsenic in fresh water fish was quantified by using the Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The unit is in μg/L.

ii) Health risk assessment

In this study, the health risk assessment was evaluated through two effects which are non-carcinogenic effects and carcinogenic effects. For non-carcinogenic effects, it use the Hazard Quotient (HQ) and for carcinogenic effects used Lifetime cancer risk (LCR) which both were to estimate the health of the respondents who exposed to arsenic through the consumption of the contaminated fresh water fish.

iii) Adults

This study recruited the adult population in both villages from the age of 18 to 59 years old.

1.6 Research objectives

1.6.1 General objective

To determine the arsenic concentration in fresh water fish and health risk assessment among adults in two areas at Kluang, Johor.

1.6.2 Specific objectives

1. To determine the socio demographic data of respondents..
2. To compare the fish frequency intake among respondents between two areas.
3. To compare the arsenic level in fish between two areas.
4. To compare the health risk assessment of respondents between two areas.
5. To determine the relationship between the freshwater fish frequency intake with health risk assessment of respondents in both villages.

1.7 Hypothesis

1. There is a significant difference of the fish frequency intake among respondents between two areas.

2. There is a significant difference of the arsenic level in fish between the two areas which higher in the polluted area than unpolluted area.

3. There is a significant difference of the health risk of respondents between the two areas which higher in the polluted area than unpolluted area.

4. There is a significant relationship between the fresh water fish frequency intake with health risk assessment of respondents in both villages.

CHAPTER 2

LITERATURE REVIEW

2.1 Arsenic properties

Arsenic (As) (**Figure 2.1**) is a common element in the earth's crust. The elemental arsenic is a gray, crystalline material which has the atomic number 33, atomic weight of 74.92 and density of 5.727 (Eisler., 1988). It has melting point of 817°C and sublimation at 613 °C. Arsenic has four valence states which are -3, 0, +3 and +5 (EPA, 2000). It showed that arsenic present in nature was ubiquitous, which means that it can freely found in the environment. It can present in air, water, soil, plants, and other living organisms. Arsenic can be divided into two types which were organic arsenic and inorganic arsenic. The inorganic arsenic was the most toxic form compared to the organic arsenic (EFSA., 2009). The International Agency for Research on Cancer (1994) had classified that inorganic arsenic is in group 1 as carcinogenic to humans. In water, arsenic can occurs in both inorganic and organic forms, and in dissolved and gaseous states (EPA., 2000). Inorganic As (V) is the most common species in water (Clement and Gary., 2005).

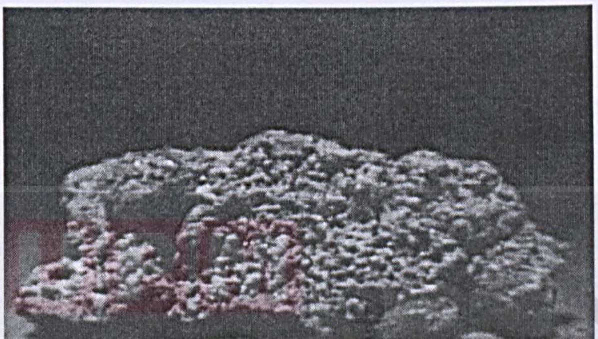

Figure 2.1: Arsenic

(Sources: World Health Organization)

2.2 Sources of arsenic pollution

Arsenic can be released to the environment in two ways which through the natural sources and the anthropogenic sources. For the natural sources, arsenic is released from the volcanoes activities and also the wind-blown soil (ATSDR., 2002). Many studies show that the arsenic that released to the environment is through the anthropogenic sources. Anthropogenic sources of arsenic include the nonferrous metal mining and smelting, agricultural activities, combustion activities and waste incineration (ATSDR., 2002; Das *et al*., 2011). The drying of concentrates in mining operations also contributes to atmospheric emissions of arsenic. In agricultural, the uses of arsenicals as pesticides and herbicides and through the burning of vegetation were some sources of the anthropogenic sources. Arsenic in soil is almost entirely in the inorganic form, except in areas with intentional organic arsenic application, where higher levels of organic compounds can be found (Saxe *et al*., 2006). In soils, pentavalent arsenic predominates due to the oxidation of trivalent arsenicals (Gong *et al*., 2001). Arsenic that found in water is mostly in the inorganic form and can be stable as both arsenite and arsenate, trivalent and pentavalent inorganic arsenicals (Saxe *et al*., 2006). EPA also states that the sources of arsenic in the drinking water include electronic wastes, glass and orchards (EPA., 2000).

2.3 Arsenic concentration in food

Food monitoring data indicated that trace concentrations of arsenic were present in all foodstuffs. Primary route of human exposure to arsenic was mainly through ingestion of foods, especially aquatic foods containing relatively high levels of arsenic. The exposure of arsenic also can happened through inhalation of air and also through dermal absorption. The U.S. Food and Drug Administration (1993) examine that in the food category which were the fish and other seafood relate with the 90% of total arsenic exposure. Donohue and Abernathy., (2002) also reported that the total arsenic in food sources of the aquatic organism such as the marine fish, shellfish and also freshwater fish. Fish and seafood can accumulate considerable amounts of organic arsenic from their environment, while the arsenic content of plants is usually determined by the arsenic content of soil, water, air, fertilizers and many more. Intake of arsenic from air and soil were usually much smaller compare from food and water.

2.4 Arsenic in fish and fish products

People are exposed to arsenic from a variety of sources including air, soil and water. Another source which may be significant of exposure to arsenic was fish consumption. Fish particularly freshwater and marine species were known to contain high concentration of arsenic (Richard J., 2002). Quantitative data on As concentration and speciation in fish tissue are sparse. Donohue and Abernathy (2002), they found very few samples of freshwater fish tissue were analyzed specifically for inorganic arsenic.

2.5 Previous studies of arsenic concentration in fish

2.5.1 International studies

In India, high concentration of arsenic in groundwater in the north-eastern states of Indian becomes a major cause of concern. Sangeeta *et al*., (2011) had done a study in that area to examine the toxicological effect and molecular changes caused by arsenic in freshwater teleost fish. It was found that the arsenic concentration was higher in the liver of the fish because it plays an important role in uptake, accumulation, bio-transformation and excretion of arsenic. Slejkovec *et al.,* (2004) reported that arsenobetaine was a major arsenical in freshwater fish, while Zheng and Hintelmann., (2004) found that arsenobetaine was present only in trace amounts in freshwater fish. This also supported by a present study, freshwater and biological samples were collected from Hayakawa River which contain a high level of arsenic from the hot springs at Mount Hakone (Shinichi et al., 2009).

2.5.2 Local studies

A present study by Alina *et al.,* (2012) had determined the level of heavy metals including arsenic in marine fish and shellfish along the Straits of Malacca. It was found that arsenic was detected for all of the marine fish and shellfish. Fathi Alhashmi *et al.*, (2011) had done a comparison of two locations for the evaluation of trace metal levels in tissues of two commercial fish species which were *Arius thalassinus* and *Pennahia anea.* It was found that the ranged of arsenic levels in the muscles of the analyzed fish was from 3.8 to 14.2 μg/g. According to published literature, the ranges of arsenic concentrations reported earlier in the muscles of Malaysian marine fish were 1.05 to 2.14 μg/g (Budiati., 2010).

2.6 Health effects

Arsenic exposure to human can cause serious problem to the health. Safiuddin *et al*., (2011) reported that the disease of melanosis and keratosis were the most common sufferings among the arsenic affected people in Bangladesh. The health effects that caused from the arsenic exposure was skin cancer which about 0.8% of the total skin of the patients. They also stated that the chronic arsenicosis contribute the role in social and economic consequences as well as through the communities which indirectly decrease the quality life (Safiuddin *et al*., 2011).

Several countries have reported that the interest in the toxicity of arsenic had been increase in the exposure through high concentration of arsenic in their drinking water and were displaying various clinical conditions. The general health effects that were associated with arsenic exposure include cardiovascular and peripheral vascular disease, developmental anomalies, neurologic and neuro-behavioural disorders, diabetes, hearing loss, portal fibrosis, hematologic disorders (anemia, leukopenia and eosinophilia) and multiple cancers. Arsenic poisoning from low dose of arsenic may show signs such dry mouth and throat, heartburn, nausea, abdominal pain and cramps also moderate diarrhea. Then, chronic low dose of arsenic ingestion may produce a mild esophagitis, gastritis or colitis with respective upper and low abdominal discomfort. Epidemiological studies have shown that the cardiovascular system is particularly sensitive to a long term ingestion of arsenic in drinking water which the health effects includes hypertension and increased cardiovascular disease mortality.

2.7 Health Risk Assessment

According to Robson *et al*., (2007), risk assessment was a function of hazard and exposure which defined as the processes of estimating the probability of the occurrence of adverse health effects over a specified time period. International Program of Chemical Safety (IPCS) had defined the concept of integrated risk assessment as 'a science-based approach that combines the processes of risk estimation for humans, biota, and natural resources in one assessment' (IPCS., 2001). Two fundamental reasons for integrated risk assessment which first, to improve the quality and efficiency of assessments through the exchange of information between human health and environmental risk assessors, and to provide more coherent inputs in the decision-making process (IPCS., 2001). In general, there are four steps for the health risk assessment which are hazard identification, exposure assessment, dose-response assessment and risk characterization.

According to the California Environment Protection Agency (EPA), the first steps for the health risk assessment was the hazard identification which to determine the types of health problems a chemical could cause by reviewing studies of its effects in humans and laboratory animals, such as headaches, nausea, eye, nose and throat irritation; or chronic diseases, such as cancer. It was the review key research to identify any potential health problems that a chemical can cause. The next step was exposure assessment which to determine the duration of human exposure to a chemical; how much the chemical they expose to and also whether the exposure are continuous or intermittent. The route of exposure to human also include in the second step of the health risk assessment. The third step was the dose response assessment which evaluated the information obtained during the hazard identification step to estimate the amount of a chemical that was likely to result in a particular health effect in humans. It divided into two effects which are cancer effects and non-cancer effects.

For chemical that cause cancer, the general assumption in risk assessment had been there are no exposures that have zero risk unless was clear evidence. In other words, even a very low exposure to a cancer-causing chemical may result in cancer if the chemical happens to alter cellular functions in a way that causes cancer to develop. Thus, even very low exposures to carcinogens might increase the risk of cancer, if only by a very small amount. The non-cancer health effects typically become more severe as exposure to a chemical increases. The goal of the dose-response assessment was to estimate the levels of exposure that pose only a low or negligible risk for non-cancer health effects. The last step in risk assessment was the risk characterizations which bring together the information develop in the previous three steps to estimate the risk of health effects in an exposed population. Risk characterization analyzed the information developed during the exposure and dose response assessments to describe the resulting health risks that are expected to occur in the exposed population.

2.8 Previous studies on Health Risk Assessment

2.8.1 International studies

A study by Monica *et al.,* (2011) had reported that in order to assess the health risks, it is necessary to identify the potential of a source to introduce risk agents into the environment, to estimate the amount of risk agents that come into contact with the human-environment boundaries, and quantify the health consequence of the exposure. Health risk assessment was an important tool that evaluated the consequence of human activities and weighs the adverse effects to public health against the contribution to economic development. Victoria., (2010) had done a study related to arsenic exposure to human through fish consumption reported that the number of cancer risk was 85 cancer cases per 100,000 populations.

2.8.2 Local studies

Aziemah *et al.,* (2012) had done a health risk assessment related to exposure of arsenic by consumption of shrimp paste. The health risk assessment was done to assess the risk faced by the respondents that consume the shrimp paste. The health risk assessment was determine through the calculation of Average Daily Dose (ADD), Lifetime Average Daily Dose (LADD), Hazard Quotient (HQ) and Lifetime Cancer Risk (LCR). Furthermore, a study by Budiati (2010) had stated that risk assessment was the scientific evaluation of the probability of occurrence and severity of known or potential adverse health effects resulting from human exposure to food borne hazards. The process consisted of hazard identification, hazard characterization, exposure assessment and risk characterization.

CHAPTER 3

METHODOLOGY

3.1 Study design

This research was a cross-sectional comparative study designed to determine the arsenic concentration in the fresh water fish and health risk faced by the respondent through the consumption of the contaminated fish. It focused on the arsenic exposure through the consumption of fish in two areas at Kluang, particularly in terms of the kinds and effects of different pollution sources of the river. Health risk assessment was also conducted to relate the fish intake for consumption with the risk of health of the residents from both areas. This study commenced from January 2013 until March 2013.

3.2 Study location

Kluang is situated in the middle state of Johor. It lies about 100 km north of Johor Bahru, east-southeast of Batu Pahat, west of Mersing and south of Segamat. Kluang is the centre of fresh water river in Malaysia. This study was conducted at Kampung Sedohok and Kampung Punjut in Kluang, Johor (**Figure 3.1**). Both villages were located nearby with two rivers, Sembrong River (**Figure 3.2**) near with Kampung Sedohok and Kahang River (**Figure 3.3**) near with Kampung Punjut in which different pollution levels based on the nearby industrial activities. The main sources of fish consumption for both villagers were from the rivers. Hence, these two villages were chosen as the location of study..

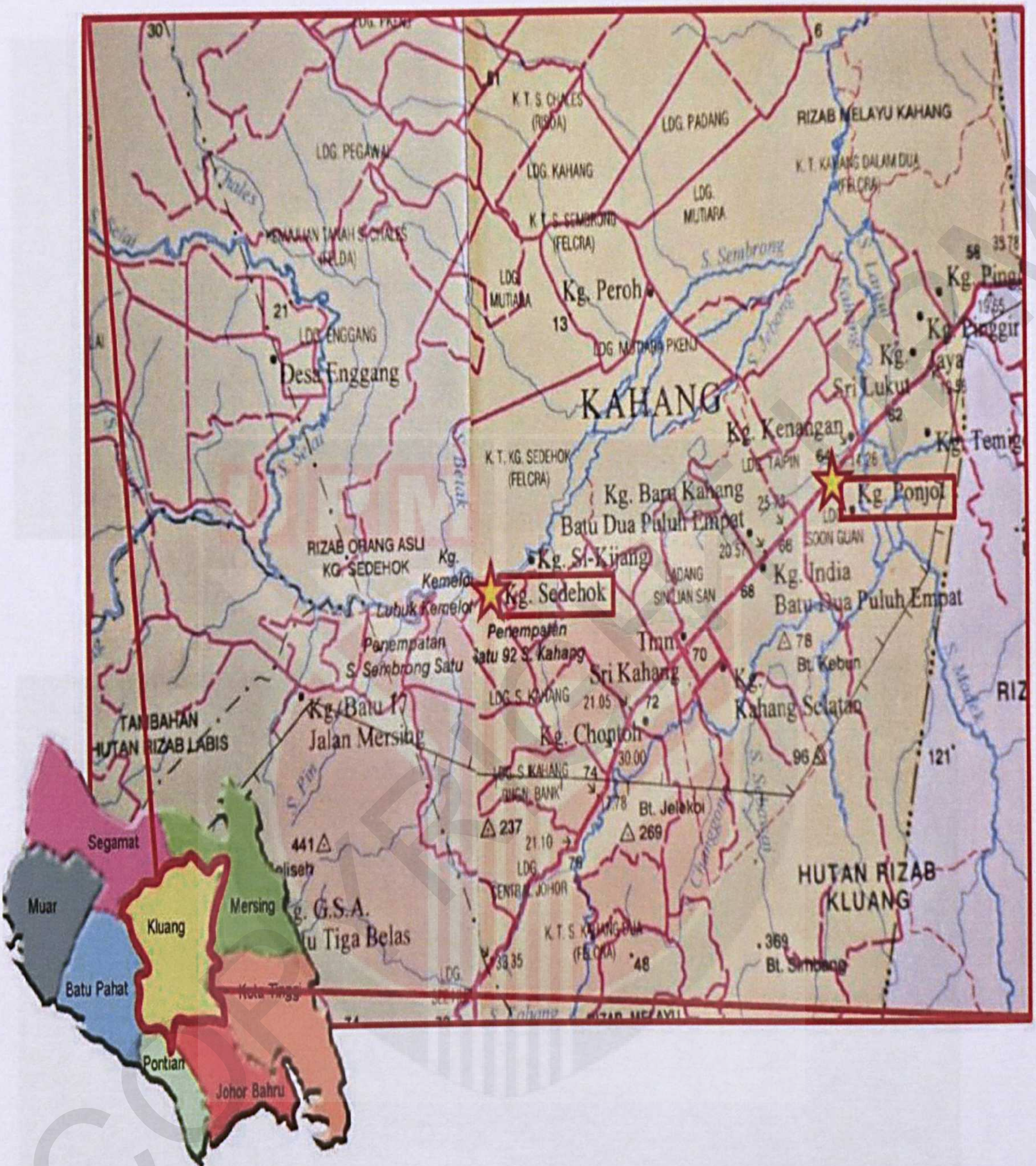

Figure 3.1: Map of study location

Figure 3.2: Sembrong River, Kluang

Figure 3.3: Kahang River, Kluang

3.3 Study Population

The residents of Kampung Sedohok and Kampung Punjut were Orang Asli villagers. Approximately there were 190 residents lived in Kampung Sedohok and 240 residents in Kampung Punjut. The main occupations of the villagers were farmer and fisherman. The fish for consumption was caught from the nearby river. The study population comprised the adult respondent.

3.4 Study Sample

The study's respondents were recruited from the two villages which were Kampung Sedohok and Kampung Punjut. The respondent were among the male and female adults which within the age of 18 years old until 59 years old and consumed the fresh water fish. This study excluded the respondent from immuno-compromised group, such as cancer patient, HIV/AIDS, transplant patient, and pregnant women.

3.5 Sample Size

The calculation of sample size for this study was based on the group comparison (two groups) using the formula below (Lu and Llewellyn., 2006).

Group Comparison ***Formula***

(Two Groups)

Ho: $\mu_1 = \mu_2$

Ha: $\mu_1 \neq \mu_2$

$$n = \frac{2\sigma^2[z_{1-\alpha/2} + z_{1-\beta}]^2}{(\mu_1 = \mu_2)^2}$$

where,

σ = estimated standard deviation (Assumed to be equal for each group)

μ_1= estimated mean (larger)

μ_2= estimated mean (smaller)

Based on Fathi *et al*., (2011), the calculation for sample size is as follow:

$\sigma = 2.34$, $\mu_1 = 14.2$, $\mu_2 = 12.58$

$$n = \frac{2\,(2.34)^2(1.96 + 0.842)^2}{(14.2 - 12.58)^2}$$

$$= 34$$

Once the sample size was calculated based on the considerations of the study's research questions and design, additional adjustments to the estimated sample size were still needed to ensure the desired target number of respondents was obtained.

Adjust for the size of the population = n / (1 + (n-1) / N)

n = Estimate sample size, 34

N = Total Population, 400

Therefore, Sample size = 34 / (1+ [(34-1) / 400])

= 31 respondents for each village

Approximately, the calculation of sample size for both villages was 62 respondents. For the purpose of this study, the researcher obtained 30 respondents for each village which total up for 60 respondents for both villages. The respondents were chosen based on the inclusive criteria. The response rate for this study was 96 percent.

3.6 Sampling Method

3.6.1 Respondent

Two location were purposely chosen to represent communities who were exposed to the polluted and less polluted river which were Kampung Sedohok and Kampung Punjut. The respondents from each location were selected through random sampling based on inclusive criteria. The inclusive criteria were adult respondent within the age of 18 to 59 years old and consumed the fresh water fish. Adult respondent had been chosen for this study because the prevalence of arsenism were increasing along with the increasing of age and extended years of residence in a particular place. The prevalence of adult residents was obviously higher than young people which make them prone to get exposed with arsenic health related problems. According to Norimah *et al.*, (2008) that stated adult population in Malaysia is between 18-59 years old. Some of the respondents were excluded from this study based on certain exclusion criteria which were person from immune-compromised group, such as cancer patient, HIV/AIDS, transplant patient, and pregnant women.

3.6.2 Freshwater fish sampling

The sampling method used for the freshwater fish sample was purposive sampling. The freshwater fish sampling took place at two different locations (**Figure 3.4**) in February 2013. The locations of sampling were based on the local fisherman fishing area. Both locations were chosen in relation with the pollution source and near with both villages of the study location. The first sampling point (FSI) was at Sembrong River. In term of the pollution level, the water quality of this location was influence by the nearest industrial activities such as manufacturing factory, wood processing factory and also pesticide usage from nearby oil palm plantation. The second sampling point was at Kahang River, it is relatively less polluted compare to Sembrong River due to the less industrial activities at that particular area.

The collection of fresh water fish samples were done by taking three (3) species of freshwater fish samples which been caught by the fisherman using net method from both river. The three (3) species were *Terubol* (*hard-lipped barb species*) (**Figure 3.6**), *Lampam Jawa* (*Javanese carp species*) (**Figure 3.5**) and *Jelawat* (*Hoeveni's slender carp species*) (**Figure 3.7**). The freshwater fish species that been caught were based on the freshwater fish frequency questionnaires answered by the respondents. The fish samples were then stored in a sterile plastic bag and put in a cool box with ice packs for temporary storage before being stored at the laboratory freezer (4°C) for further analysis. Arsenic concentrations in the freshwater fish samples were measured by using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) to determine the level of arsenic in the samples.

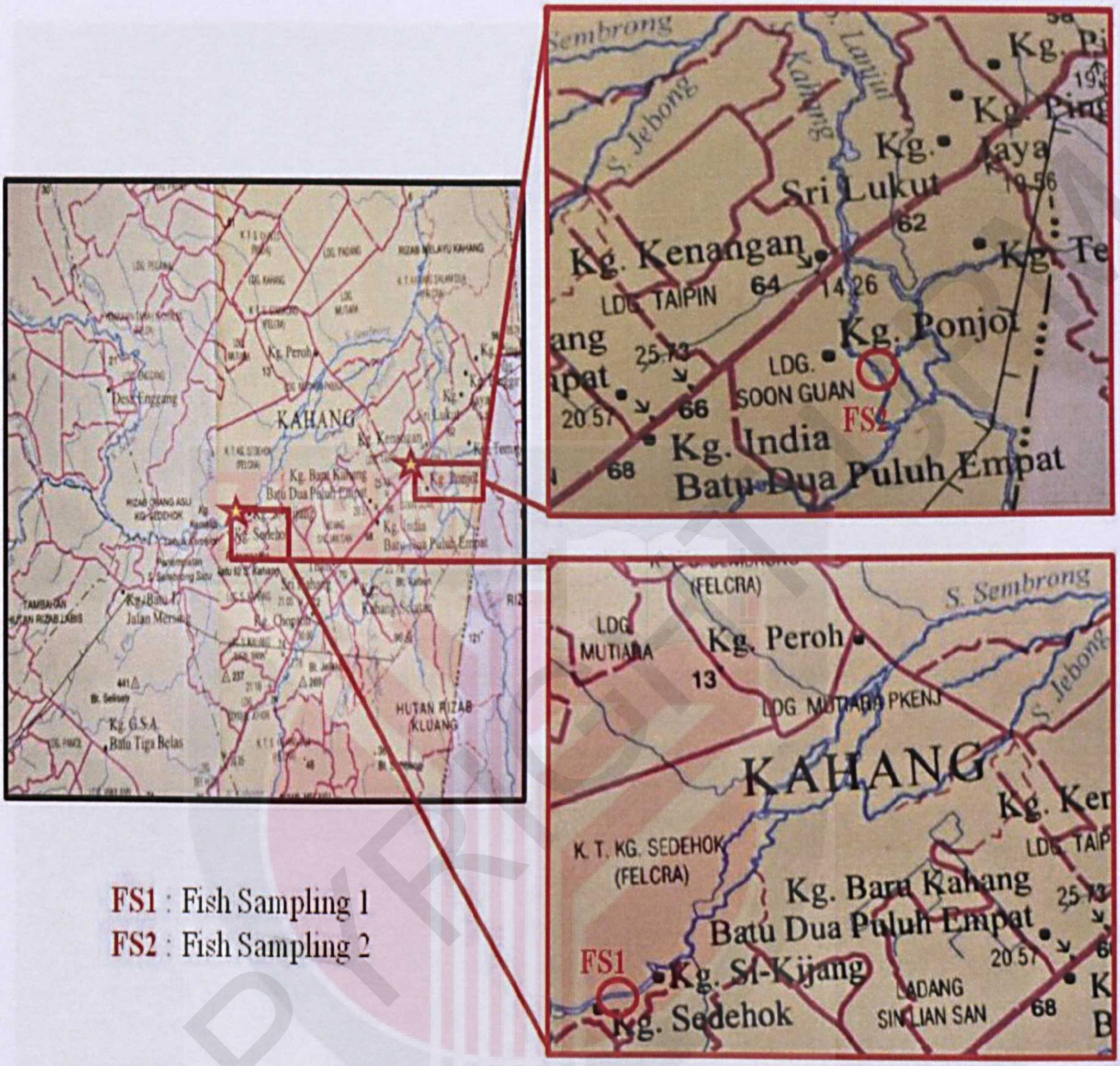

Figure 3.4: Sampling locations of the freshwater fish for both villages

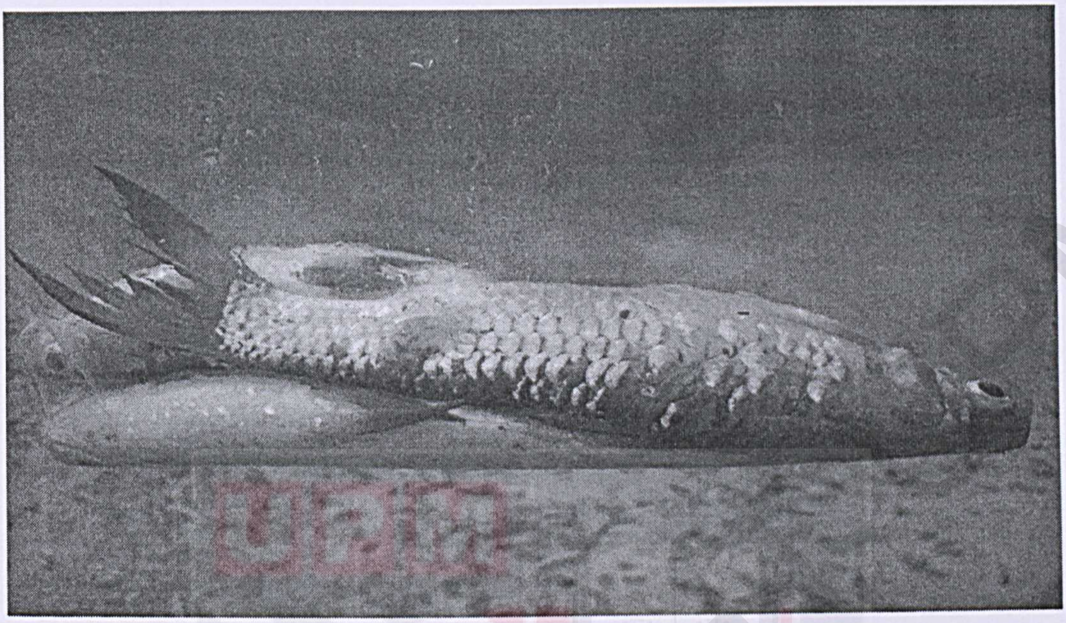

Figure 3.5: ***Lampam Jawa*/Javanese carp species *(Puntius gonionotus cyprinidae*)**

Figure 3.6: ***Terubol*/hard-lipped barb species (*Osteochillus hasseltii cyprinidae*)**

Figure 3.7: ***Jelawat*/Hoeveni's slender carp species *(Leptobarbus hoevenii cyprinidae)***

3.7 Data collection and Instrumentation

3.7.1 Data Collection Flowchart

Figure 3.8A and **Figure 3.8B** showed the flowcharts of the data collection and data analysis process of this study in which to determine the arsenic concentration in the freshwater fish and the health risk assessment among the adults at both villages in Kluang. The first step of the data collection was to obtain the approval from Universiti Research Ethics Committee (JKEUPM), Universiti Putra Malaysia (**Appendice 1**). After the approval was obtained, pre-survey was carried out at the study locations to gather some information about the facilities in the community. The survey was assissted by Jabatan Kemajuan Orang Asli (JAKOA), Kluang and Fishery Department of Kluang. Researcher had a meeting with the head chief of both villages to discuss further about the research study and explained about the procedure of the study. The researcher obtained the permission from the head chief of both villages to carry out the study in their villages.

Pre-test questionnaires were carried out in order to test the questionnaires so it can be understand by the respondents and suitable for the study population. The pre-test was conducted at Kampung Tanah Runtuh, Kluang. After validation of the questionnaires, the data was collected through the distribution of the questionnaires to all the residents in both villages. The questionnaires included the socio demographic of respondents, health status, anthropometry measurement, food frequency intake and others possible sources of arsenic exposure. Freshwater fish samples were also collected at the study location to fulfill the research objectives. All the samples were brought to the laboratory to be analyzed. The Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was used in the detection of arsenic concentration in freshwater fish samples.

Universiti Research Ethics Committee (JKEUPM),

Universiti Putra Malaysia

↓

Pre-survey

1. Fresh water fish information was obtained from the Fisheries Department of Kluang, Johor.

2. Both villages information were obtained from the Jabatan Kemajuan Orang Asli (JAKOA), Kluang, Johor.

2. Discussion with the head chief of both villages (Kampung Sedohok and Kampung Punjut) to explain the research study and to obtain the permission from the head chief to conduct the research study

3. Pre-test questionnaires

↓

Data Collection

1. Respondent Information sheets (**Appendice 3**) were distributed and explained to the respondents

2. Distribution of questionnaire (Food Frequency Questionnaire)

3. Collection of freshwater fish samples

Figure 3.8A: Flowchart of data collection

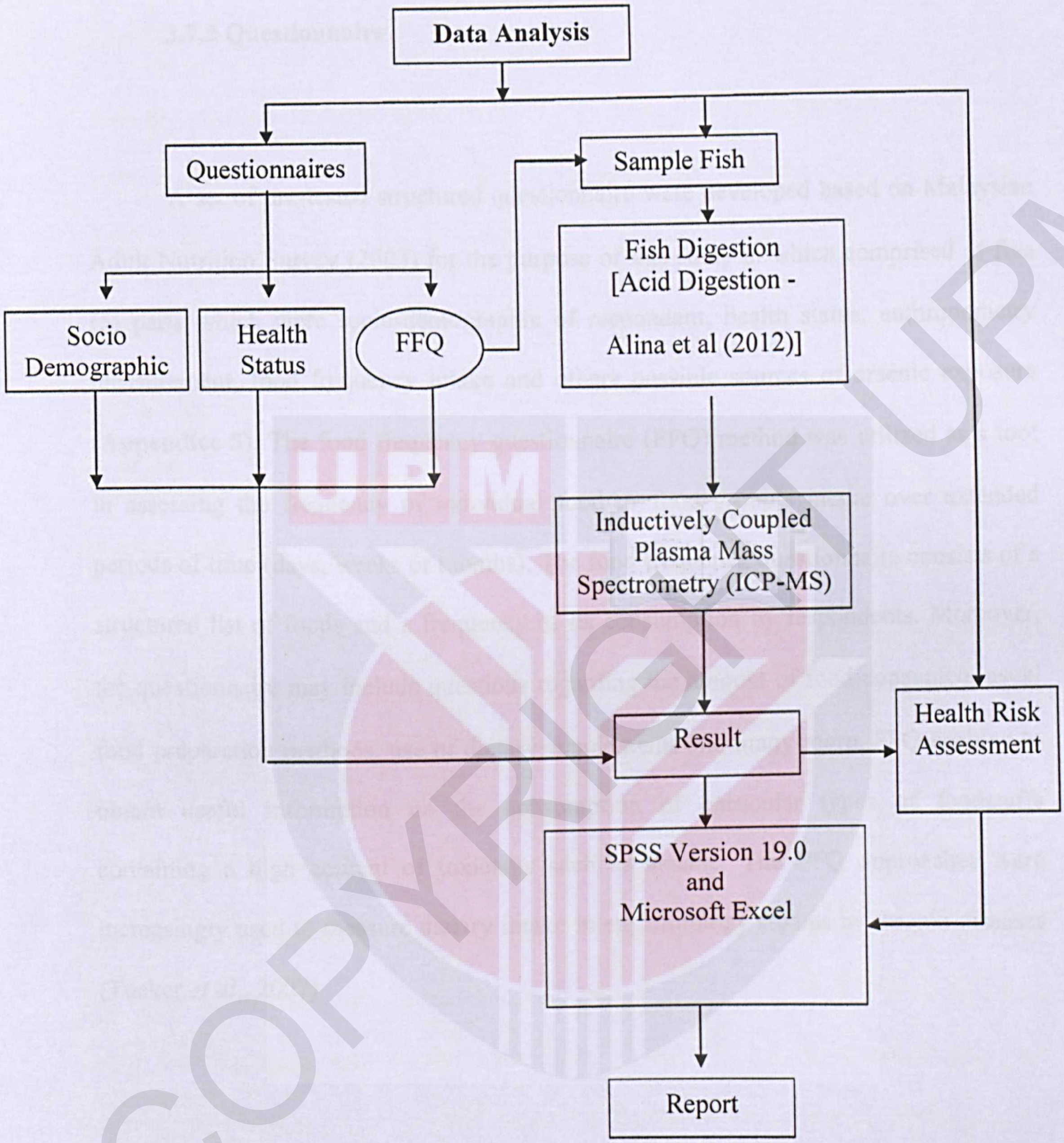

Figure 3.8B: Flowchart of data analysis

3.7.2 Questionnaire

A set of pre-tested structured questionnaire were developed based on Malaysian Adult Nutrition Survey (2003) for the purpose of this study in which comprised of five (5) parts which were socio-demographic of respondent, health status, anthropometry measurement, food frequency intake and others possible sources of arsenic exposure (**Appendice 5**). The food frequency questionnaire (FFQ) method was utilized as a tool in assessing the frequency of individual food or food group's intake over extended periods of time (days, weeks or months). The food frequency questionnaire consists of a structured list of foods and a frequency of its consumption by respondents. Moreover, the questionnaire may include questions regarding the amount of food consumed, usual food preparation methods, use of dietary supplements and many more. FFQ enabled to obtain useful information on the consumption of particular types of foodstuffs containing a high content of toxicants such as arsenic. The FFQ approaches were increasingly used to measure dietary intake in epidemiology studies in chronic diseases (Tucker *et al*., 2007).

Part 1: Socio demographic details

This part consists of the personal details of the respondents. It includes the information such as sex, ethnicity, marital status, educational level, occupation, individual income and household income.

Part 2: Health status

The health status part was regarding the general health condition of the respondents and health symptoms of As poisoning.

Part 3: Anthropometry measurement

This part consists of weight and height information of the respondent. Then, the body mass index (BMI) of the respondent was calculated using this formula:

$$BMI = Weight\ (Kg) / Height\ (m^2) \times Height\ (m^2)$$

Part 4: Food Frequency intake information

The main purpose of this part is to determine the most frequently consume fish. This information is needed to choose the fish species to be sampled for As determination in fish. Other purpose was to determine other possible food sources of arsenic. This includes the food intake of their daily consumption such as vegetables, fruits and others.

Part 5: Others possible sources of arsenic exposure

This part consists of other sources of arsenic exposure to the respondents. It includes the sources from drinking water, smoking habit, occupation related with pesticide and alcohol consumption by the respondent.

3.8 Sample Analysis

3.8.1 Fish sample preparation and digestion

Three species of fish samples were caught from both villages according to the sampling locations by the fisherman. The fish samples were stored in a cool box and transported to the laboratory for fish digestion (Alina M. *et al.,* 2012). The total length (cm) and weight (gram) of the fish samples (**Table 3.2**) were measured using ruler and analytical balance before dissection (**Figure 3.9**). The list of equipments and chemical used in the analysis was shown in **Table 4.1**. The samples were thoroughly washed with deionised water after removing the scales, skin, muscle and other organs. The muscle portions were taken which only used in this study for further processing and analysis. The fish samples were dissected with sterilized stainless steel equipment. The dissected part, the muscle portion were dried in an oven at 75°C for two days in acid-washed Petri dish until a constant weight was obtained. When the samples reached the constant weight, samples were allowed to cool at room temperature and crushed into a fine powder using pestle and mortar. Then, each sample, 0.5 gram (dry weight) powdered form of fish muscle in triplicate was weighed and 5 ml of 25% HNO_3 was added to each triplicate samples and the mix was stored at room temperature for one day. After that, the samples were heated using a conventional vacuum oven at the temperature of 65°C to 80°C for 8 hours until a clear solution was obtained. After clear solution was obtained, the samples were filtered through 0.45 µm Whattman filter paper. The digested samples were measured and finally made up with 0.25 M of HNO_3 to 25 ml acid washed volumetric flask. Next, all the digested samples were put into a clean polyethylene bottle for the analysis with Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The concentration of arsenic in the fish samples were measured with the ICP-MS. All the glassware and plastics used in the preparation of samples were soaked overnight in 10% (v/v) nitric acid and rinsed with distilled and deionized water and dried before being used.

Table 3.1: List of equipments and chemical used for fish preparation and digestion

Materials and chemical	
Petri dish	Analytical balance
Stainless Steel Knife	Spatula
Pestle and Mortar	Pipette
Alcohol Swab	0.45 µm Whattman filter paper
Beaker	Conventional Vacuum Oven
Volumetric flask	Measuring cylinder
Aluminium foil	Nitric acid (HNO_3)
Ruler	

Freshwater fish sampling, capture and stored.

↓

Length (cm) and weight (g) of samples are measure.

↓

The samples washed, remove scales and dissect.

↓

Dried in oven (75°C) and homogenize

↓

0.5 g (dry weight) fish muscles are weight

↓

Mix with 5 ml 25% HNO_3 and stored in room temperature (1 day)

↓

Heat 65°C- 80°C (8 hours) using conventional vacuum oven

↓

The digested samples are filtered and made up with 0.25 M HNO_3 to 25 ml in acid washed volumetric flasks

↓

Put into a clean polyethylene bottle for the analysis with ICP-MS.

Figure 3.9: Flow chart of the sample preparation and digestion

3.8.2 Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Arsenic in freshwater fish sample was extracted by using acid digestion method and then analyze by using Inductively Coupled Plasma - Mass Spectrometry (ICP-MS) with unit µg/L (**Figure 3.10**). The principle of Inductive Coupled Plasma (ICP) was where atom or ions of the element present in the sample absorb energy, causing excitation of their electrons to unstable energy states. When these atoms or ions return to their stable ground-state configuration, they emit light of specific wavelengths characteristic of the elements. ICP was argon plasma that can heat the sample to a very high temperature in the range 5500 to 8000^{o}C. Sample was injected as an aerosol, and transported onto the ICP by argon, which was also used as a carrier gas. The emitted light was separated according to its wavelength and measures. For ICP-MS it was method combine an ICP with a quadrapole mass spectrometer to separate the ions produced. High energy ICP generates single charged ions from the atoms of the elements present in the sample. Such ions were now directed onto the mass spectrometer, separated, and measured according to their mass-to-charge ratio. ICP-MS was also known as an analytical technique that performs elemental analysis with excellent sensitivity. The typical detection limit for ICP-MS was 0.01-1 microgram per liter in solution. ICP-MS can perform very fast whereby it can detect up to 60 elements in a 2 minutes scan.

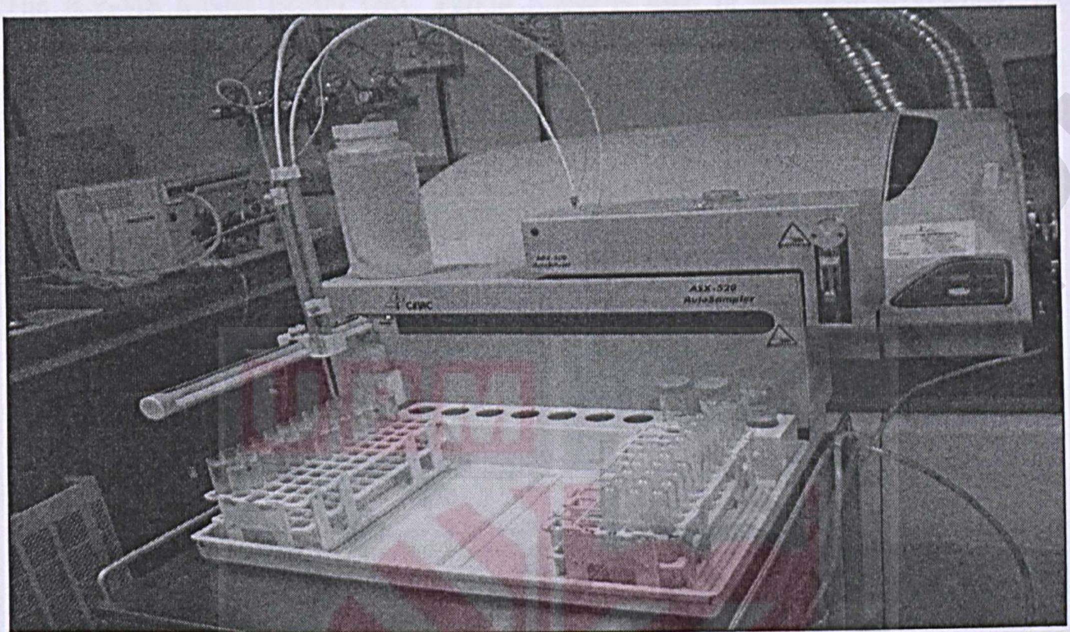

Figure 3.10: Inductively Coupled Plasma-Mass Spectrometry (ICP-M

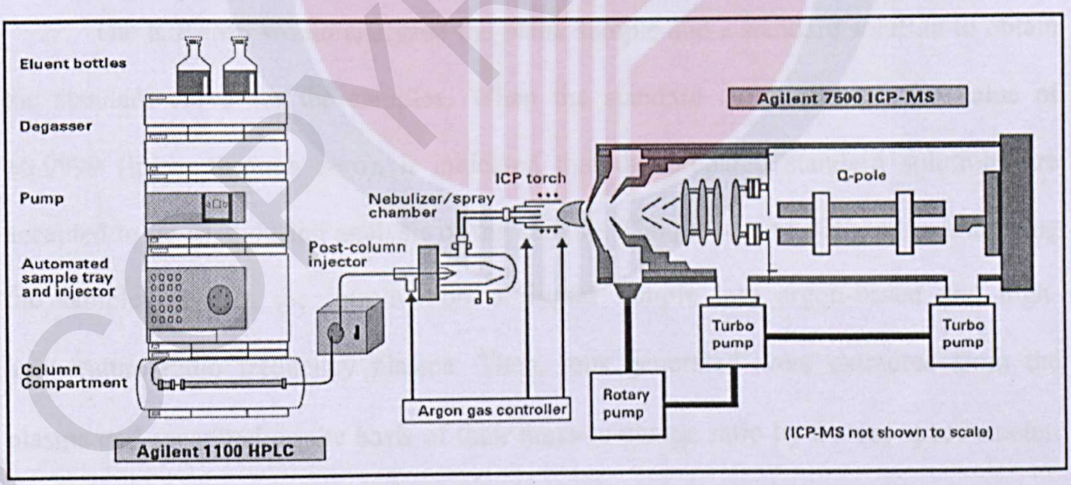

Figure 3.11: ICP-MS Coupling System

Figure 3.12 shows the step and procedure of the arsenic analysis using ICP-MS. The ICP-MS Model ELAN DRC Perkin Elmer is used in detection of arsenic. A minimum sample volume of 10 to 20 ml is needed for the analysis. However, it is essential that the sample is representative and precaution steps must be taken to avoid the contamination of the sample. A standard solution of 10 000 ppb is prepare. Then, the standard solution is dilute into 5 ppb, 10 ppb, 20 ppb, 50 ppb, 100 ppb and 300 ppb. Next, all the samples were labelled properly and poured into the cuvette tube and placed in the rack before being analyzed by ICP-MS. Then, 6 prepared standard solutions (5 ppb, 10 ppb, 20 ppb, 50 ppb, 100 ppb and 300 ppb) which from the same rack and one blank sample (deionized water) were placed together to be analyzed in order to get the standard curve of the samples.

The ICP- MS would analyzed the blank sample and a standard solution to obtain the standard curve for the samples. When the standard curve showed the value of ±0.9999 (linear through zero), it indicated that the prepared standard solutions are accepted to be used during analysis of the sample. The process of ICP-MS in analyzing the sample includes the introduction of liquid sample into argon-based and high-temperature radio frequency plasma. Then, ions generated were extracted from the plasma and separated on the basis of their mass-to-charge ratio by a mass spectrometer. The technique is useful for multi-element determination and solid samples and samples containing precipitates must be digested prior to analysis.

Standard solution for ICP-MS was prepared by using stock solution into 5 ppb, 10 ppb, 20 ppb, 50 ppb, 100 ppb and 300 ppb by using this formula:

$$M_1V_1 = M_2V_2$$

↓

The deionized ultrapure water was poured into the 100 ml volumetric flask, followed with the stock solution (5 ppb, 10 ppb, 20 ppb, 50 ppb, 100 ppb and 300 ppb). The fish samples were poured into 10 ml test tube and placed in a zigzag arrangement on the rack provided.

↓

All samples were run by using ICP-MS.

↓

All results were printed out

Figure 3.12: Steps and procedure of ICP-MS

3.8.3 Anthropometry Measurement

The anthropometry equipments used in this study were Seca digital platform scale and the Portable Seca-body meter (**Figure 3.13**). The Seca digital platform was used to measure the weight of the respondent. The scale was placed on a stable and flat surface when taken the measurement of the respondent. The unit measurement was in kilogram (kg). The Portable Seca-body meter was used to measure the height of the respondent. The Seca body meter was taped to a vertical wall which perpendicular to the level of the floor and then used to measure the height of the respondent. The unit measurement was in centimetre (cm).

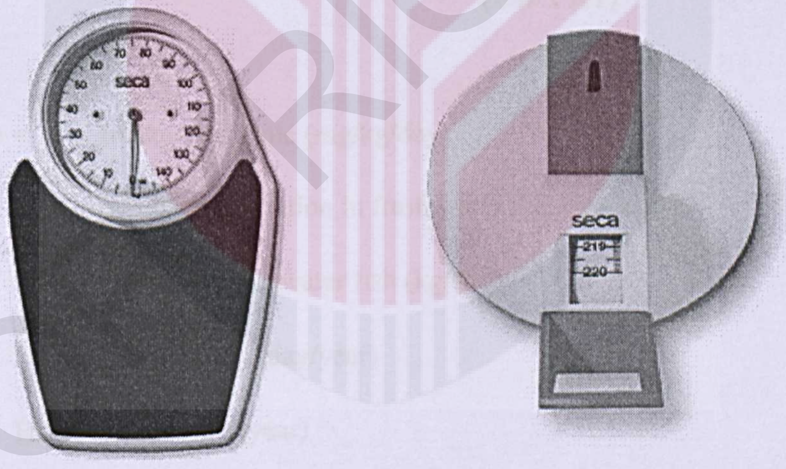

Figure 3.13: Seca digital platform scale and Portable Seca body meter

3.9 Health Risk Assessment (HRA)

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

The health risk assessment is evaluated through Average Daily Dose (ADD) or Lifetime Average Daily Dose (LADD) which to determine the intake of arsenic in food by using the following formula:

$$\text{ADD / LADD} = \frac{(\text{C x IR x EF x ED})}{(\text{BW x AT})}$$

Where,

ADD / LADD = Intake of arsenic (mg/kg/day)

C = Total arsenic concentration in fresh water fish (mg/kg)

IR = Ingestion rate of fresh water fish (kg/day)

EF = Exposure frequency (day/year)

ED = Exposure duration (year)

BW = Body weight (kg)

AT = Averaging time (days)

3.9.2 Hazard Quotient (HQ) and Lifetime Cancer Risk (LCR)

A. Hazard Quotient (HQ)

The Hazard Quotient (HQ) indicated for health risk of non-carcinogenic health effects encountered by the respondents. The Hazard Quotient (HQ) present in 2 categories which are acceptable and clearly unacceptable. Then, it will be calculate for the non-carcinogenic risk using the following formula:

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

Where,

Hazard Quotient (HQ) = Non-cancer Hazard Index of a health effect from intake of arsenic

ADD = Average Daily Dose (mg/kg/day)

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

RfD arsenic – $3x10^{-4}$ mg/kg/day (USEPA., 2007)

After the calculation, the Hazard Quotient value is compared with the following values of risk acceptability for non-carcinogenic health effects in **Table 3.2**. In cases where the non-cancer hazard quotient does not exceed unity which is $HI < 1$, it is assume that no chronic risks are likely to occur (Mallory., 2010).

Table 3.3: The risk acceptability for non-carcinogenic health effect (USEPA., 2007)

Hazard Quotient (HQ)	
>1	Unacceptable
<1	Acceptable

B. Lifetime Cancer Risk (LCR)

The Lifetime Cancer Risk (LCR) indicates for health risk of carcinogenic health effects encounter by the respondents. The Lifetime Cancer Risk (LCR) are presented in 3 categories which were clearly acceptable, acceptable and clearly unacceptable. The calculations of the LCR were using the following formula:

$$\text{Lifetime Cancer Risk (LCR)} = \text{LADD} \times q^*$$

Where,

LCR = Upper limit cancer risk

LADD = Lifetime Average Daily Dose (mg/kg/day)

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

Inorganic arsenic cancer slope factor is 1.5 mg/kg/day (USEPA., 2007)

The Lifetime Cancer Risk value obtained from the calculation will be referred to the following **Table 3.3** which to determine the risk acceptability for carcinogenic health effects.

Table 3.4: The risk acceptability for carcinogenic health effect (USEPA, 2007)

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

3.10 Quality Control

3.10.1 Standard Operating Procedure (SOP)

The analysis of fish samples were using ICP-MS model Perkin Elmer followed the standard operating procedures in order to prevent any analytical error while analyzing the arsenic concentration in fish samples, all the preparation and procedures was conducted according to the guideline book for the Perkin-Elmer ICP-MS provided by the manufacturer. Next, the weighing scales and height scales used in the survey were standardized and calibrated to ensure the validity of the measurement.

3.10.2 Pre Test of Questionnaire

Pre-test questionnaire was conducted for 10% from the respondents total sample size before data collection to ensure that every question includes in the questionnaire was understandable and suitable with the respondent. The questionnaire was tested on a random sample of the community to assess its clarity of the questionnaires. This test was conducted at the residents of Kampung Tanah Runtuh, Kluang Johor. A total of 10 respondents were recruited which was 10% of the total study population. The value of the cronbach's alpha reliability test was 0.86.

3.11 Data Analysis

Data that collected from this study were analyzed using Statistical Package for Social Science (SPSS) version 19.0 and Microsoft Excel. Three types of statistical analyses were used which were descriptive, comparison and correlation analysis. The descriptive analysis was to describe the distribution of socio demographic of respondent in both villages. Comparison analysis between group were using Mann- Whitney U test, in order to determine the difference of arsenic between groups concentration and also the health risk of the respondents for both villages. Correlation analysis was used to assess the relationship between the fresh water fish frequency intake and the health risk assessment of respondents for both villages using Spearman's rho correlation test.

3.12 Ethical Issues

The study protocol was reviewed and approved by the University Research Ethics Committee (JKEUPM), Universiti Putra Malaysia (**Appendice 1**). The respondent's consent were obtained and signed by respondents before the commencement of the data collection. All respondents were given a choice whether to continue participating in the study or pull out at any time they choose to do so. The information of the respondents, especially their identities will remain confidential and only used for the purpose of this study.

CHAPTER 4

RESULTS

This study was conducted at two areas which were Kampung Sedohok and Kampung Punjut situated in Kluang, Johor. Both villages were Orang Asli settlements. The study commenced from January until March 2013. The respondents included male and female adult residents. Questionnaires were distributed to the respondents participated in this study. The most highly consumed freshwater fish samples were caught by the fisherman using net method from both villages. Health risk was determine through the indication of Average Daily Dose (ADD), Hazard Quotient (HQ), Lifetime Average Daily Dose (LADD) and Lifetime Cancer Risk (LCR).

4.1 Socio demographic of the respondents

The socio demographic of respondents in both villages were shown in **Table 4.1A** and **Table 4.1B**. **Table 4.1A** shows the gender, religion, marital status, education level and job sector of the respondents in both villages. Both villages showed that more male respondents selected compared to the female respondents. Most of the respondents in both villages practices animism as their religion. Approximately 80% of the respondents in both villages were already married and the remaining was single. The education level showed that most of the respondents in both villages had a basic education level in which they went to kindergarden or primary school. For the job sector, the results showed that in Kampung Sedohok, most of the respondents were not working which include housewife and retires. In Kampung Punjut, most of the respondents were self-employed. The least job sector for both villages was employed which categorized into private sector and government sector.

Table 4.1B shows the distribution of age, household income, body mass index (BMI) and number of household. The result showed that the youngest respondent participated in this study was 18 years old and the oldest was 57 years old. The highest age group of the respondents from Kampung Sedohok was between 20 to 29 years old while for Kampung Punjut was between 30 to 39 years. A total of 63 percent of the respondents from both villages had the same household income below the poverty line index (PLI) of RM 760. The poverty line index (PLI) of Malaysia for household income in rural areas was RM760. The range of household incomes for both villages was between RM 100 to RM2000. The body mass index (BMI) of the respondents for both villages showed that most of them were normal. The result also showed that the obese respondents were higher in Kampung Punjut with 87 percent compared to Kampung Sedohok with 60 percent. Most of the respondents in both villages have a household number less than 5 people in a house and ranged from 2 to 12 people.

Table 4.1A: The socio demographic information for Kampung Sedohok and Kampung Punjut

Variable	Kampung Sedohok (n=30)			Kampung Punjut (n=30)				
	Freq.	%	Mean ± SD	Freq.	%	Mean ± SD	X^2	p
Gender:								
Male	19	63.3		17	56.7			
Female	11	36.7		13	43.3		0.27	0.598
Religion:								
Muslim	1	3.3		0	0			
Animism	29	96.7		30	100		1.01	0.313
Marital Status:								
Single	4	13.3		6	20			
Married	26	86.7		24	80		0.48	0.488
Education Level:								
Not Attend School	7	23.3		11	36.7			
Kindergarden/Primary	19	63.3	1.7 ± 0.9	12	40	1.5 ± 1.3	5.42	0.247
Secondary/Higher	4	13.4		7	23.3			
Job Sector:								
Employed	4	13.3		1	3.3			
Self-employed	11	36.7	1.8 ± 0.7	15	50	2.0 ± 0.3	3.17	0.365
Not working/Others	15	50		14	46.7			

Table 4.1B: The socio demographic information for Kampung Sedohok and Kampung Punjut

Variable	Kampung Sedohok (n=30)			Kampung Punjut (n=30)				
	Freq. (%)	Mean ± S.D	Range	Freq. (%)	Mean ± S.D	Range	Z	p
Age groups (years):		32.5 ± 8.36	18 – 56		33.9 ± 11.7	19 - 57	-0.059	0.953
18 - 19	1 (3.3)			3 (10)				
20 – 29	13 (43.3)			9 (30)				
30 – 39	10 (33.4)			11 (36.7)				
40 – 49	5 (16.7)			2 (6.7)				
50 – 59	1 (3.3)			5 (16.6)				
BMI (kg/m^2):		23.3 ± 5.4	16.0 – 37.5		28.1 ± 13.6	17.0 - 91.7	-0.448	0.654
Underweight	4 (13.3)			3 (10)				
Normal	17 (56.7)			14 (46.7)				
Overweight	6 (20)			5 (16.7)				
Obese	3 (10)			8 (26.7)				
Household Income:		551.6 ±367	200 - 1300		725 ± 482	100 - 2000	-1.641	0.101
≤ 760	19 (63.3)			19 (63.3)				
> 760	11 (36.7)			11 (36.7)				
Household :		5 ± 2	2 – 13		5 ± 2	1 - 12	-2.446	0.014
≤ 5	26 (86.7)			18 (60)				
> 5	4 (13.3)			12 (40)				

4.2 Health status information

Table 4.2 shows that the health problems of the respondents for both villages. Based on the result obtained, there were 3 major health problems faced by the respondents for both villages which were joint pain, skin disease such as itchiness and rash and shortness of breath. It shows that the percentage of the respondents that experience the three major health problems were slightly higher in Kampung Sedohok compared to the Kampung Punjut. A percentage of 70 percent of the respondents in Kampung Sedohok experienced the joint pain compared with respondents in Kampung Punjut with 57 percent. The others health problems are in a small percentage that experience by the respondents from both villages.

Table 4.3 shows the health symptoms that experienced by the respondents in which related to the arsenic exposure. The results show that there are three higher percentage of health symptoms that faced by the respondents which are headache, lethargy and muscle fatigue. Respondents in Kampung Sedohok experienced muscle fatigue and lethargy slightly higher compare to respondents in Kampung Punjut. While for headache, a percentage 73 percent of the respondents in Kampung Punjut experience the symptoms which higher compare to respondents of Kampung Sedohok with a percentage of 67 percent. Vomiting, skin allergic, asthma, depression, loss of appetite and diarrhea are the others health symptoms experienced by the respondents in both villages but in a small proportion.

Table 4.2: Health problems of the respondents

Health problems	Kampung Sedohok (n=30)		Kampung Punjut (n=30)	
	Yes Freq. (%)	No Freq. (%)	Yes Freq. (%)	No Freq. (%)
Urination problems	7 (23.3)	23 (76.7)	2 (6.7)	28 (93.3)
Bone problems	8 (26.7)	22 (73.3)	12 (40)	18 (60)
Kidney disease	3 (10)	27 (90)	2 (6.7)	28 (93.3)
Joint pain	21 (70)	9 (30)	17 (56.7)	13 (43.3)
Bone disease	4 (13.3)	26 (86.7)	4 (13.3)	26 (86.7)
Skin disease (itchness, eczema, rash)	11 (36.7)	19 (63.3)	9 (30)	21 (70)
Lung problems (Inflammation)	2 (6.7)	28 (93.3)	3 (10)	27 (90)
Shortness of breath	13 (43.3)	17 (56.7)	11 (36.7)	19 (63.3)
Lung cancer	2 (6.7)	28 (93.3)	0	30 (100)
Anemia	2 (6.7)	28 (93.3)	0	30 (100)
Hypertension	8 (26.7)	22 (73.3)	9 (30)	21 (70)

Table 4.3: Health symptoms related to the exposure of arsenic that experienced by the respondents

Health problems	Kampung Sedohok (n=30)		Kampung Punjut (n=30)	
	Yes Freq. (%)	No Freq. (%)	Yes Freq. (%)	No Freq. (%)
Vomiting	5 (16.7)	25 (83.3)	6 (20)	23 (80)
Headache	20 (66.7)	10 (33.3)	22 (73.3)	8 (26.7)
Lethargy	21 (70)	9 (30)	16 (53.3)	14 (46.7)
Skin allergic	7 (23.3)	23 (76.7)	6 (20)	24 (80)
Asthma	2 (6.7)	28 (93.3)	8 (26.7)	22 (73.3)
Depression	7 (23.3)	23 (76.7)	3 (10)	27 (90)
Loss of appetite	12 (40)	18 (60)	7 (23.3)	23 (76.7)
Muscle fatigue	19 (63.3)	11 (36.7)	10 (33.3)	20 (66.7)
Diarrhea	12 (40)	18 (60)	16 (53.3)	14 (46.7)

4.3 Freshwater fish frequency intake of respondents

Figure 4.1 shows the fresh water fish frequency intake of respondents at Kampung Sedohok and Kampung Punjut. The fresh water fish frequency intakes were categorized into two parts which were frequent includes everyday and 3 – 6 per times per week. The other category was less frequent which includes 1 – 2 times per week, 2 – 4 times per month, once a month and seldom. A total of 82 percent of the respondents in Kampung Sedohok and 70 percent of the respondents in Kampung Punjut were frequently consumed fresh water fish.

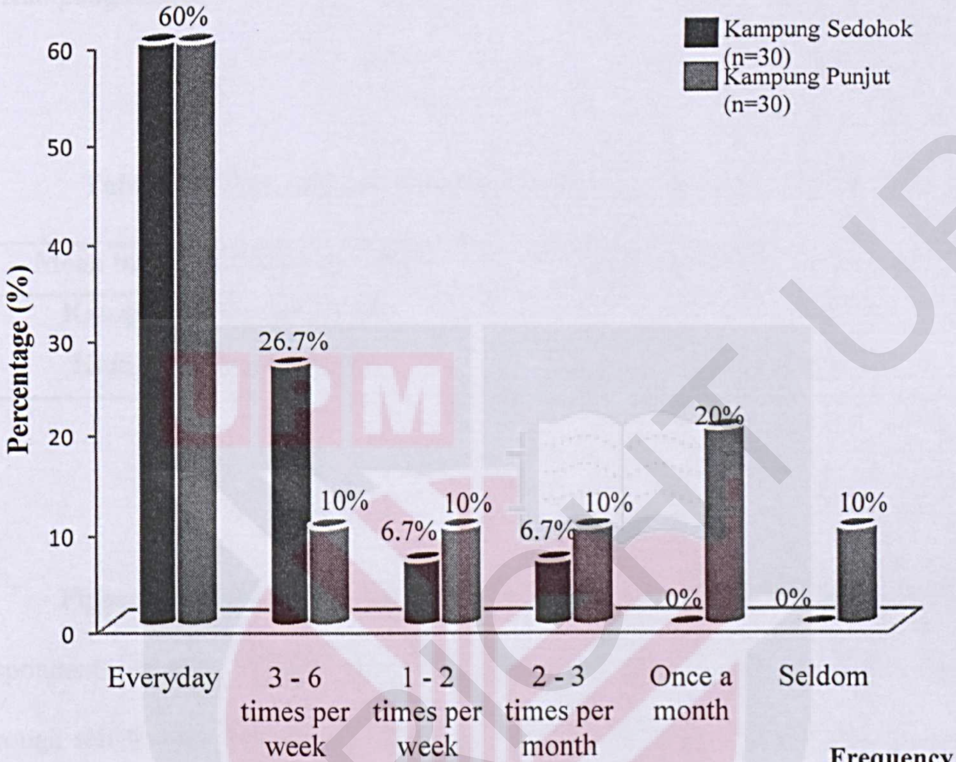

Figure 4.1: The fresh water fish frequency intake among the respondents of Kampung Sedohok and Kampung Punjut

Table 4.4 shows the mean intake of the fresh water fish for both villages. Based on the results obtained, it shows that the mean intake of fresh water fish among the respondents in Kampung Sedohok was slightly higher when compared with respondents in Kampung Punjut.

Table 4.4: The mean intake of the fresh water fish of both villages

Mean intake of freshwater fish	Mean ± Standard Deviation
Kampung Sedohok (n=30)	0.114 ± 0.087
Kampung Punjut (n=30)	0.110 ± 0.088

Figure 4.2 shows the sources of freshwater fish for the consumption by the respondents of both villages. The respondents got their source of fresh water fish through self-fishing, bought at market and from the fish cage. Most of the respondents from both villages got their source of freshwater fish through self-fishing due to the occupation of the respondents are mostly working as a fisherman.

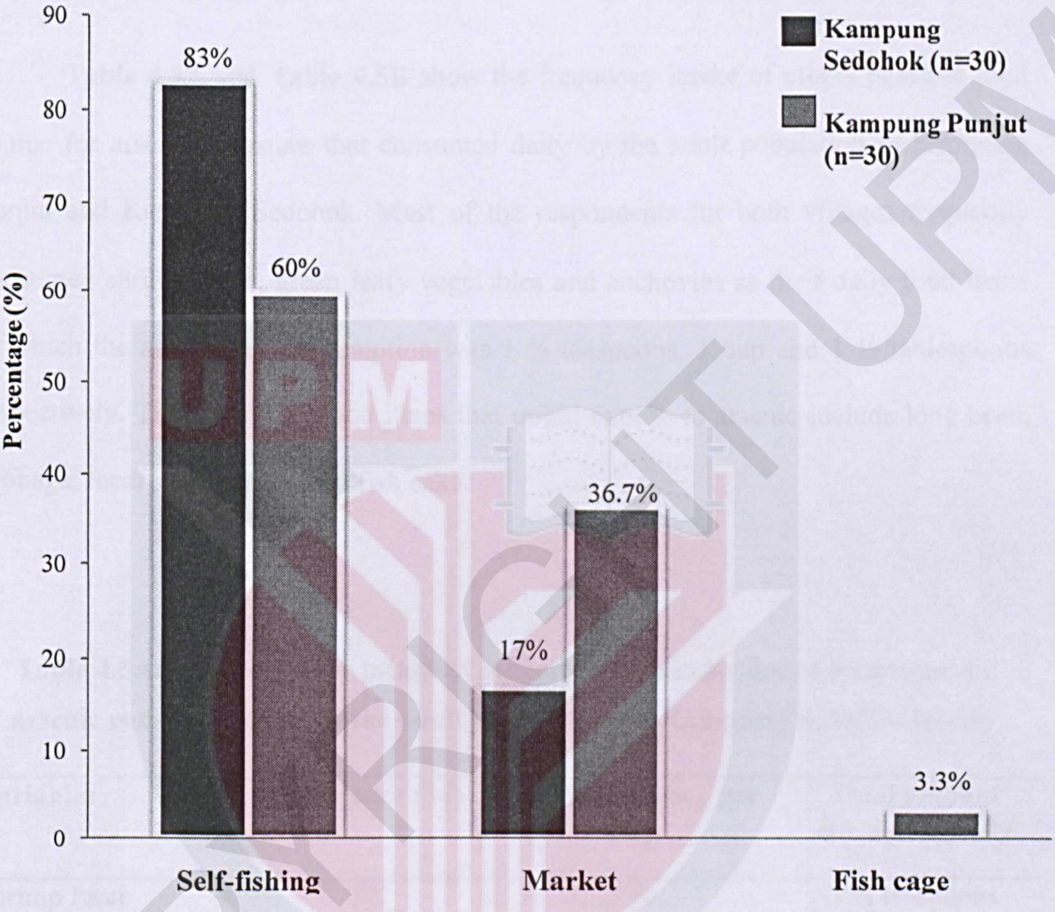

Figure 4.2: The sources of fresh water fish for consumption by the respondents

4.4 Other possible exposures of Arsenic (As)

Table 4.5A and **Table 4.5B** show the frequency intake of others possible food source for arsenic exposure that consumed daily by the adult population in Kampung Punjut and Kampung Sedohok. Most of the respondents for both villages frequently consumed shrimp paste, green leafy vegetables and anchovies as their daily food items in which the average of consumption was 1 ½ teaspoons, 1 cup and 1 ½ tablespoons respectively. The remaining food items that might expose to arsenic include long bean, cabbage, local salad (*ulam*) and fish cake.

Table 4.5A: The frequency intake of others possible exposure of food items to arsenic consumed daily by the adult population for Kampung Sedohok (n=30)

Variables	Prevalence (%)	Mean frequency per day	Total amount consumed daily
Shrimp Paste	57	3.18	1 ½ teaspoons
Green leafy vegetable	50	2.13	1 cup
Anchovies	47	2.36	1 ½ tablespoons
Local Salad (*ulam*)	37	2.18	1 cup
Fish cake	30	1.5	1 piece
Cabbage	20	2	1 cup

Table 4.5B: The frequency intake of others possible exposure of food items to arsenic consumed daily by the adult population for Kampung Punjut (n=30)

Variables	Prevalence (%)	Mean frequency per day	Total amount of consumed daily
Anchovies	50	2.13	1 cup
Shrimp paste	47	3.14	1 ½ teaspoons
Green leafy vegetable	43	2.07	1 cup
Long bean	43	1.92	1 cup
Cabbage	27	2.25	1 cup
Local salad (*ulam*)	23	1.85	1 cup

Table 4.6A and **Table 4.6B** show the frequency intake of others possible exposure of food items to arsenic that consumed weekly by the adult population for both villages. Most of the respondents in Kampung Sedohok consumed long bean, canned fish and fried fish crackers (*Keropok lekor*) as their top highly consumed food items weekly with average of consumption of 1 ½ tablespoons, 1 piece and 3 pieces respectively. Fish cake, peanut and fried fish crackers (*Keropok lekor*) were the top highly food items consumed weekly by the adult respondents in Kampung Punjut.

Table 4.6A: The frequency intake of others possible exposure of food items to arsenic consumed weekly by the adult population for Kampung Sedohok (n=30)

Variables	Prevalence (%)	Mean frequency per weekly	Total amount Consumed weekly
Long bean	60	1.83	1 ½ tablespoons
Canned fish	53	1.31	1 piece
Fried fish crackers (*Keropok lekor*)	53	1.43	3 piece
Baby Corn	50	1.7	1 piece
Local Salad (*ulam*)	47	2.07	1 ½ tablespoons
Anchovies	43	2.71	1 ½ tablespoons
Ground nut	43	1.85	1 cup
Peanut	43	1.62	1 cup
Prawn (wet)	40	2.08	2 pieces

Table 4.6B: The frequency intake of others possible exposure of food items to arsenic consumed weekly by the adult population for Kampung Punjut (n=30)

Variables	Prevalence (%)	Mean frequency per weekly	Total amount consumed weekly
Fish cake	57	1.529	1 piece
Peanut	43	1.46	1 cup
Fried fish crackers (*Keropok lekor*)	40	1.33	3 pieces
Cabbage	40	1.33	1 cup
Tapioca	37	1.36	1 piece
Anchovies	33	1.9	1 ½ tablespoons
Canned fish	33	1.5	1 piece
Cuttle fish (wet)	33	2.08	2 pieces

The exposure of Arsenic (As) was not only through the fresh water fish consumption or others food items but also from other possible exposure which were shown in **Table 4.7**. In term of water source, most of the respondents in Kampung Sedohok used well water as the main water source, while in Kampung Punjut, all of the respondents used tap water as the their water source. A total number of 16 respondents in Kampung Sedohok and 14 respondents in Kampung Punjut were smokers. Most of the respondents for both villages do not consume alcohol and do not have experience with job related to the exposure of pesticide.

Table 4.7: Others possible exposure to arsenic

Variables	**Kampung Sedohok (n=30)**		**Kampung Punjut (n=30)**	
	Freq.	**Percent (%)**	**Freq.**	**Percent (%)**
Water sources				
Tap water	1	3.3	30	100
Well water	29	96.7	0	0
Smoking habit				
Yes	16	53.3	14	46.7
No	14	46.7	16	53.3
Alcohol consumption				
Yes	7	23.3	8	26.7
No	23	76.7	22	73.3
Job related exposure to pesticide				
Yes	13	43.3	9	30
No	17	56.7	21	70

4.5 Arsenic (As) concentration in fresh water fish samples

Figure 4.3 shows the arsenic concentration detected in the freshwater fish samples for both villages which a total of six samples from three types of freshwater fish that been caught from the selected rivers in Kluang, Johor. **Table 4.8** showed the three types of fresh water fish were Lampam Jawa, Terubol and Jelawat that had been caught from selected rivers nearest with both villages which were from Sembrong River (Kampung Sedohok) and Kahang River (Kampung Punjut). The maximum permitted level of arsenic based on Malaysian Food Act 1983 and Food Regulation 1985 was 1 mg/kg. The fish samples were analyzed using Inductively Coupled Plasma Mass spectrometry (ICP-MS) after the digestion process with nitric acid. All the fish samples were reported in µg/L. **Table 4.9** shows the parameters for the calculation of arsenic concentration from ICP-MS. These parameters were used in order to convert the raw result data which obtained from the ICP-MS to the actual reading of the arsenic concentration in the fresh water fish.

Table 4.8: The total length (cm) and weight (gram) of the fish samples

Local fish name/ General Name	Fish Scientific Name	Sample ID	Qty	Total length (cm)	Total weight (gram)	Habitat
***Jelawat* / Hoeveni's Slender carp**	*Leptobarbus hoevenii cyprinidae*	FK2a	1	18.4	110.0	Kahang River
		FS2b	1	20.8	120.0	Sembrong River
***Lampam Jawa* / Javanese Carp**	*Puntius gonionotus cyprinidae*	FS3a	1	19.2	120.0	Sembrong River
		FK3b	1	23.0	2000.0	Kahang River
***Terubol* / Hard-lipped barb**	*Osteochillus hasseltii cyprinidae*	FS1a	1	19.6	140.0	Sembrong River
		FK1b	1	24.0	240.0	Kahang River

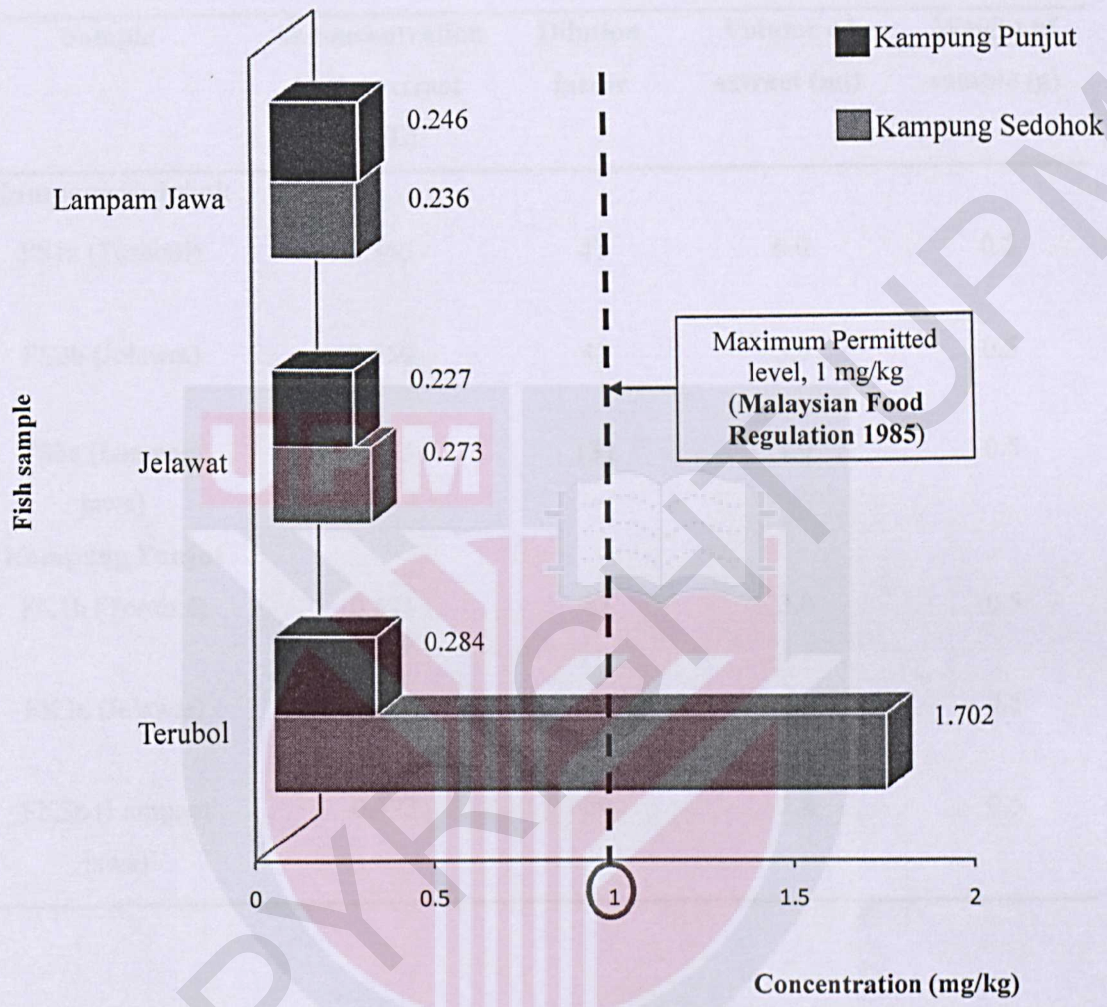

Figure 4.3: The arsenic concentration in fresh water fish samples in both villages

Table 4.9: The parameters for calculation of Arsenic concentration from ICP-MS

Sample	As concentration in the extract (μg/L)	Dilution factor	Volume of extract (ml)	Weight of sample (g)
Kampung Sedohok				
FS1a (Terubol)	3.346	41	6.0	0.5
FS2b (Jelawat)	0.550	42	5.9	0.5
FS3a (Lampam jawa)	0.475	131	1.9	0.5
Kampung Punjut				
FK1b (Terubol)	0.571	83	3.0	0.5
FK2a (Jelawat)	0.456	86	2.9	0.5
FK3b (Lampam jawa)	0.493	89	2.8	0.5

Figure 4.4 showed the clear view of the difference of mean concentration of arsenic in the fresh water fish for both villages. The result showed that the mean concentration of arsenic in fresh water fish for both villages were below the maximum permitted level which was 1 mg/kg. But, the mean concentration of arsenic in fish was higher in Kampung Sedohok compared to Kampung Punjut.

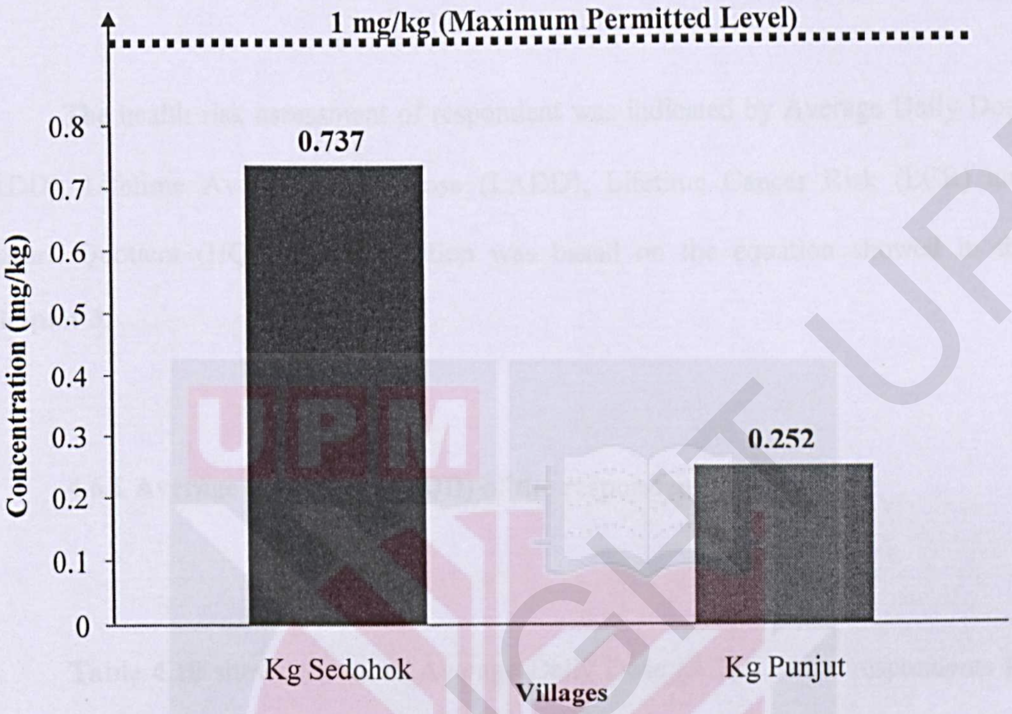

Figure 4.4: The mean concentration of arsenic in fresh water fish in both villages

4.6 Health risk assessment of respondents

The health risk assessment of respondent was indicated by Average Daily Dose (ADD), Lifetime Average Daily Dose (LADD), Lifetime Cancer Risk (LCR) and Hazard Quotient (HQ). The calculation was based on the equation showed in the **Chapter 3.**

4.6.1 Average Daily Dose (ADD) of the respondents

Table 4.10 shows the mean Average Daily Dose (ADD) of the respondents for both villages which were Kampung Sedohok and Kampung Punjut. The mean value of the Average Daily Dose (ADD) for Kampung Sedohok was slightly higher compared to Kampung Punjut.

Table 4.10: Mean of Average Daily Dose (ADD) of the respondents

ADD (mg/kg/day)	Mean ± Standard Deviation
Kampung Sedohok (n-30)	$2.247 \times 10^{-7} \pm 0.417 \times 10^{-7}$
Kampung Punjut (n=30)	$0.319 \times 10^{-7} \pm 0.10 \times 10^{-7}$

4.6.2 Lifetime Average Daily Dose (LADD) of the respondents

Table 4.11 shows the mean Lifetime Average Daily Dose (LADD) of the respondents for both villages which were Kampung Sedohok and Kampung Punjut. The mean value of the Lifetime Average Daily Dose (LADD) for Kampung Sedohok was slightly higher compared to Kampung Punjut.

Table 4.11: Mean of Lifetime Average Daily Dose (LADD) of the respondents

LADD (mg/kg/day)	Mean ± Standard Deviation
Kampung Sedohok (n=30)	$1.027 \times 10^{-7} \pm 0.198 \times 10^{-7}$
Kampung Punjut (n=30)	$0.173 \times 10^{-7} \pm 0.045 \times 10^{-7}$

4.6.3 Lifetime Cancer Risk (LCR) for carcinogenic health effects

Table 4.12 shows the Lifetime Cancer Risk (LCR) that indicated for the health risk for carcinogenic health effects for both villages. Lifetime Cancer Risk (LCR) was categorized into three categories which were clearly acceptable, acceptable and clearly unacceptable. The results show that all the respondents in both villages were fall under clearly acceptable risk in which lifetime cancer risk value was more than 10^{-6}.

Table 4.12: Health risk for carcinogenic health effects for Kampung Sedohok (KS) and Kampung Punjut (KP)

Lifetime Cancer Risk (LCR)		Frequency	Percent (%)	Mean ± SD	Range
KS					
$<10^{-6}$	Clearly acceptable	30	100	$1.54 \times 10^{-7} \pm 0.29 \times 10^{-7}$	2.6×10^{-9} – 1.29×10^{-7}
10^{-6}-10^{-4}	Acceptable				
$>10^{-4}$	Cleary unacceptable				
KP					
$<10^{-6}$	Clearly acceptable	30	100	$2.58 \times 10^{-8} \pm 0.68 \times 10^{-8}$	3.954×10^{-12} – 3.2×10^{-7}
10^{-6}-10^{-4}	Acceptable				
$>10^{-4}$	Cleary unacceptable				

4.6.4 Hazard Quotient (HQ) for non-carcinogenic health effects

Table 4.13 shows the Hazard Quotient (HQ) which indicated the health risk for non-carcinogenic health effects encountered by the respondents for both villages. Hazard Quotient (HQ) was categorized into 2 categories which were acceptable and unacceptable. Based on the result obtained, all of the respondents in both villages fall under acceptable condition.

Table 4.13: Health risk for non-carcinogenic health effects for Kampung Sedohok (KS) and Kampung Punjut (KP)

Hazard Quotient (HQ)		Frequency	Percent (%)	Mean ± SD	Range
KS					
<1	Acceptable	30	100	$7.36 \times 10^{-4} \pm 1.39 \times 10^{-4}$	3.17×10^{-6} – 5.53×10^{-3}
>1	Unacceptable				
KP					
<1	Acceptable	30	100	$1.08 \times 10^{-4} \pm 0.3 \times 10^{-4}$	7.5×10^{-9} – 1.46×10^{-3}
>1	Unacceptable				

4.7 Comparison of the fish frequency intake of respondents between the two areas

Table 4.14 showed the comparison of fish frequency intake among respondents between the two areas which were Kampung Sedohok and Kampung Punjut. The data distribution of fish frequency intake between the two areas were analyzed using Chi-Square Test of Contingency because both of the data were categorical data in which to determine the difference of fish frequency intake of respondents between the two areas. It was found that there was no significant difference between fish frequency intake of respondents between the two areas.

Table 4.14: The comparison of the fish frequency intake of respondents between the two areas

Variable	Fish Frequency Intake (FFI)			X^2	p value
	Frequent	Less Frequent	Total		
Village					
Kampung Sedohok	26	4	30	2.455	0.117
Kampung Punjut	21	9	30		
Total	47	13	60		

X^2 = Chi-Square test of Contigency

4.8 Comparison of the arsenic level in fresh water fish between the two areas

Table 4.15 showed the comparison of the arsenic level in fresh water fish between the two areas which were Kampung Sedohok and Kampung Punjut. The data distribution of arsenic level in fresh water fish for both villages were analyzed using normality test which is Shapiro-wilk test due to the sample size that less than 100. It was found that the data for Kampung Sedohok was not normally distributed while the data for Kampung Punjut was normally distributed. Hence, Mann-Whitney U test was used to determine whether there is a significant difference between the arsenic concentrations in fresh water fish of the two villages. It was found that there was no significant difference between the arsenic concentration in fresh water fish between Kampung Sedohok and Kampung Punjut.

Table 4.15: The comparison of the mean arsenic level in fresh water fish between the two areas

Arsenic concentration (mg/kg)	Median	Mean rank	Z	p value
Kampung Sedohok	0.273	4.00	-0.655	0.513
Kampung Punjut	0.246	3.00		

Z = Mann-Whitney U test

4.9 Comparison of the health risk assessment of respondents between the two areas

Table 4.16 shows the comparison of health risk assessment of the respondents which hazard quotient (HQ) for non-carcinogenic health effects and Lifetime cancer risk (LCR) for carcinogenic health effects between the two areas in Kluang. The data distribution of health risk assessment of respondents for both villages was analyzed using Shapiro-wilk normality test due to the sample size that less than 100. It was found that HQ data for both villages were not normally distributed. Hence, Mann-Whitney U test was used to determine whether there is a significant difference between the health risks for hazard quotient (HQ) for non-carcinogenic health effects between the two villages. It was found that the health risk of respondents for HQ in Kampung Sedohok was significantly higher ($p<0.001$) as compared to Kampung Punjut. Hence, there was a significant difference between the health risks for hazard quotient for non-carcinogenic health effects between the two villages.

For the LCR data, it was found that LCR data for both villages were not normally distributed. Hence, Mann-Whitney U test was used to determine whether there is a significant difference between the health risks for lifetime cancer risk (LCR) for carcinogenic health effects between the two villages. Based on the result obtained, it was found that the health risk of respondents for LCR in Kampung Sedohok was significantly higher ($p<0.001$) as compared to Kampung Punjut.

Table 4.16: The comparison of hazard quotient (HQ) for non-carcinogenic health effects and Lifetime cancer risk (LCR) for carcinogenic health effects of the respondents between the two areas

Variables	Median	Mean Rank	IQR	Z	p value
Hazard Quotient (HQ)					
Kampung Sedohok	7.47×10^{-5}	39.60	6.11×10^{-4}		
Kampung Punjut	2.36×10^{-5}	21.40	4.62×10^{-4}	-4.036	< 0.001*
Lifetime Cancer risk (LCR)					
Kampung Sedohok	1.72×10^{-8}	40.23	1.87×10^{-7}		
Kampung Punjut	0.36×10^{-8}	20.77	7.8×10^{-8}	-4.317	< 0.001*

***significant at p<0.001; Z = Mann-Whitney U Test**

4.10 Relationship between frequency intake of fresh water fish and the health risk assessment of the respondents in two areas

4.10.1 Relationship between frequency intake of fresh water fish and Hazard Quotient (HQ) for non-carcinogenic health effects of respondents in two areas

Table 4.17 shows the relationship between the fish frequency intake and health risk for Hazard Quotient (HQ) for non-carcinogenic health effects of the respondents in the two villages. The data distribution was analyzed Shapiro-wilk normality test due to the sample size that less than 100. It was found that the data for both villages was not normally distributed. Hence, the relationship was analyzed using the Spearman's rho test. Based on the result, it was found that there was a significant relationship between the fish frequency intake and the Hazard Quotient (HQ) for non-carcinogenic health effects of the respondents in Kampung Sedohok. However, there was no significant relationship between the fish frequency intake and the Hazard Quotient (HQ) for non-carcinogenic health effects of the respondents in Kampung Punjut. The correlation coefficient, r for Kampung Sedohok shows a strong relationship between the frequency intake and health risk of hazard quotient (HQ) of the respondents.

Table 4.17: The relationship between frequency intake of fresh water fish and Hazard Quotient (HQ) for non-carcinogenic health effects of respondents in two areas

Variables	Kampung Sedohok		Kampung Punjut	
	r	p	r	p
Frequency intake of fresh water fish	0.790	< 0.001*	0.023	0.905

***significant at p< 0.001; r = Spearman's rho test**

4.10.2 Relationship between frequency intake of fresh water fish and Lifetime Cancer Risk (LCR) for carcinogenic health effects of respondents in two areas

Table 4.18 shows the relationship between the fish frequency intake and the Lifetime Cancer Risk (LCR) for carcinogenic health effects of the respondents in Kampung Sedohok and Kampung Punjut. The data was analyzed with Shapiro-wilk normality test due to the sample size that less than 100. It was found that LCR data for both villages were not normally distributed. Hence, the results were analyzed using Spearman's rho test. The results showed that there was a significant relationship between the fish frequency intake and the Lifetime Cancer Risk (LCR) for carcinogenic health effects of the respondents in Kampung Sedohok. However, there was no significant relationship between the fish frequency intake and the Lifetime Cancer Risk (LCR) for carcinogenic health effects of the respondents in Kampung Punjut. The correlation coefficient, r for Kampung Sedohok shows a strong relationship between freshwater fish frequency intake and health risk of Lifetime Cancer Risk (LCR) of the respondents.

Table 4.18: The relationship between fish frequency intake and Lifetime Cancer risk (LCR) for carcinogenic health effects of respondents in two areas

Variables	Kampung Sedohok		Kampung Punjut	
	r	p	r	p
Frequency intake of fresh water fish	0.787	< 0.001*	0.031	0.870

***significant at p<0.001; r = Spearman's rho test**

CHAPTER 5

DISCUSSIONS, CONCLUSION AND RECOMMENDATION

The data of socio demographic of the respondents of the study location was important in order to assess the distribution of the demographic and the characteristic of the respondents that being studied. A total number of 30 respondents from each village were recruited based on the inclusive criteria to participate in this study. Based on the sample size calculation, the total sample should be 31 respondents from each village. Since, there were only 30 respondents for each village recruited for this study thus the response rate was 96.7 percent.

5.1 Socio demographic of respondents

In this study, the respondents were among the adult populations within the age of 18 to 59 years old. Norimah *et al*., (2008) stated the adult population in Malaysian is within the age 18 to 59 years. This strongly proven by Wan Abdul Manan *et al*., (2012) that the eligible adult respondents based on Malaysian adults aged within 18 to 59 years old. The prevalence of exposure to arsenic was increasing along with the increasing of age and also the extended period of residence or the respondents in a particular location. The number of respondents that participated in this study was slightly higher among the male respondents compared to the female respondents for both villages. This was due to the timing of the data collection that was carried out in the evening when all of the male respondents were back from work. For the female respondents, most of the female respondents were housewives and they were busy with the household chores activities. In term of religion, most of the respondents for both villages were animism.

For the job sector of the respondents, the results showed that the most of the respondents were not working. This is due to the age constraint of the respondents in Kampung Sedohok where most of them were senior citizen who already retired or not working and some of the female respondents were housewives. Most of the respondent for both villages have a normal body mass index (BMI) in which range between 18.5

and 24.9. However, both villages still have respondents that were obese which higher percentage in Kampung Punjut compared to Kampung Sedohok.

5.2 Freshwater fish frequency intake of the respondents

The results showed that most of the respondents for both villages frequently consumed the fresh water fish. This is expected as reported by Zuraini *et al*., (2006) which revealed that 60 to 70 percent of protein needs were fulfilled by the consumption of fishes in Malaysia. Fish is one of the most widely distributed organism in the aquatic environment and it considered as one of the main protein sources of food for human (Rashed., 2001). Besides protein, fish is also high in essential fatty acids (EFAs), known as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which are important to the diet of human. Furthermore, the consumption of fatty fish is recommended by the nutritionist due to the mean that it can prevent cardiovascular diseases (Domingo *et al*., 2007). Freshwater fish are similar to the marine fishes which both play an important role in determining the resident's diet (Ahmad *et al*., 2009). Fish plays a particularly important role in the diet of the rural population.The frequency intake of the fresh water fish is important to be determined in order to indicate the Average Daily Dose (ADD) and Lifetime Average Daily Dose (LADD) of each respondent. Average Daily Dose (ADD) is important to estimate the non-carcinogenic health risk faced by the respondents. Based on USEPA (2007), it was defined that Average Daily Dose (ADD) is dose rate averaged over a pathway specific period of exposure in which expressed as a daily dose on a per unit body weight basis. The Lifetime Average Daily Dose (LADD) is the estimation of dose to an individual averaged over a lifetime of 70 years which used in the indication of carcinogenic health risk.

5.3 Other possible exposures of Arsenic (As)

Respondents from both villages might also be exposed to arsenic through other sources such as food products other than freshwater fish. Based on the result, it shows that most of the respondents from both villages were frequently consumed shrimp paste, green leafy vegetable and anchovies. Previous local study of the toxicology evaluation of some Malaysian locally processed raw food products found that shrimp paste were contaminated with certain heavy metal (Shariff *et al.*, 2008). Based on study by Aziemah *et al.,* (2012), it was found that arsenic was detected in the shrimp and exceeded the maximum level of the arsenic concentration stated by the Malaysian Food Act 1983 and Food Regulations 1985. The source of arsenic in the shrimp paste was influenced by marine organism which through the species differences, size of organism and human activities (Attar *et al.*, 1992).

Other than that, vegetables such as leafy vegetable and long bean were frequently consumed by the respondents in both villages. Wang *et al.,* (2008) revealed that the levels of heavy metals in vegetables and soils have correlation to the health risk of people. A study by Bernhard *et al*., (2004) on heavy metal in soils and crops in Peninsular Malaysia found that the addition of phosphate fertilizers to the agricultural soils which resulted in increasing of heavy metal concentration such as arsenic. From the interviewed session with some of the respondents of both villages, they indicated that the sources of their vegetables consumption were from their own agricultural farming. Hence, this could be the other sources of arsenic exposure based on the used fertilizers for the crop. Anchovy was also frequently consumed by the respondents in both villages. It also might be one of the sources of arsenic exposure. Base on study by Suzilla *et al*., (2012), it was found that arsenic was detected in anchovy sauce samples and the concentration of arsenic was exceeding the safe level of consumption. Other than that, people were also exposed to arsenic through water supplies such as drinking water, cigarettes smoking and exposure to pesticide. It was found that drinking water was a potential source of arsenic in dietary exposures especially the population that used well water. Pesticide used for agricultural such as crops also contain chemical that contribute to arsenic contamination. Smoking was also another possible exposure to arsenic.

All these informations show that the arsenic exposure can be from the contamination of certain types of sources such as food items, social behavior and sources of water as well.

5.4 Arsenic concentration in freshwater fish

Based on the results obtained, it was found that arsenic was detected in all the freshwater fish samples. The mean arsenic level in Kampung Sedohok (0.737 mg/kg) was slightly higher compared to Kampung Punjut (0.252 mg/kg). The freshwater fish was caught from the two rivers in Kluang, Johor which were Sembrong River nearest with Kampung Sedohok and Kahang River nearest with Kampung Punjut. Both rivers were the sources of the freshwater fish consumption for both villages. The arsenic level was higher in Kampung Sedohok due to the intensive industrial activities from manufacturing factories and agricultural activities near the Sembrong River (**Figure 5.1**). Furthermore, the factory released their effluent to the river (**Figure 5.2**). Based on observation, there was also a wood processing factory near the Sembrong River (**Figure 5.3**). There was a study by Brooks., (2009) indicated that arsenic were used in pesticides in agricultural and also wood preservatives. Arsenic trioxide used in wood preservation has accounted for 86 to 90 percent of total U.S. arsenic consumption. Wood treated with chromated copper arsenate (CCA), known as "pressure-treated wood," has been used widely to protect utility poles, building lumber, and foundations from decay and insect attack. According to the Environmental Quality Report (2010) had reported that Sembrong River as one of the slightly pollute river which classified in Class III. Based on Paquin *et al*., (2003), the coastal or river waters were contaminated by the dumping of industrial wastages. The metals will then accumulated in the river waters also will affect humans through the direct consumption of water or through consuming the contaminated organisms like fishes. When compare to Kampung Punjut (Kahang River), there were less industrial activities located nearest to the river. According to the Environmental Quality Report (2010) Kahang River is one of the clean river which classified in Class II. Based on Mason *et al*., (2000), the efficiency of metal uptake from contaminated water and food may differ in relation to ecological needs, metabolism, and the contamination gradients of water, food and sediment, as well as salinity and temperature. Hence, the difference of arsenic level in fresh water fish between the two villages due to the different environment condition in term of the availability of the anthropogenic activities.

Figure 5.1: Manufacturing factory near the Sembrong River

Figure 2: Effluent released to Sembrong River

Figure 2: Wood processing factory near the Sembrong River

According to the Malaysian Food Regulation 1985, the maximum level for arsenic detected in food was 1 mg/kg. There were less data of arsenic levels in freshwater fish in muscle tissues from Malaysian study. Bhupander Kumar *et al*., (2011) had stated that the muscle tissue of fish is the frequently used for analysis due to its major target tissues for metal storage and it is also the main edible part of the fish. Tuzen (2003) stated that the observed differences in the metal concentration in fish can be explained by the fact that the concentrations of the metals depend to a great extent on species, sex, biological cycle, and on the part of the fish analyzed. Moreover, ecological factors might also contribute to variations in the metal concentrations in fishes such as season and temperature.

Overall, Arsenic (As) was detected in all freshwater fish samples and only one sample was exceeded the maximum permitted level of As concentration in food which stated by the Malaysian Food Regulations 1985 (1 mg/kg). The rest of the freshwater samples were below the maximum permitted level of As concentration.

5.5 Health risk assessment

The health risk assessment was indicated through the Average Daily Dose (ADD) and Lifetime Average Daily Dose (LADD) in which to determine the intake of arsenic in food. The value of ADD and LADD indicated for Hazard Quotient (HQ) for health risk of non-carcinogenic health effects and Lifetime Cancer Risk (LCR) for health risk of carcinogenic health effects encountered by the respondents. Based on the results, it was found that the mean of Average Daily Dose (ADD) for Kampung Sedohok was slightly higher compared to Kampung Punjut. The HQ values showed that for all of the respondents in both villages were less than one (<1). This means that the ADD value for all of the respondents were below the reference dose of arsenic which was 3×10^{-4}. Hence, this indicated that the respondents possess an acceptable risk condition. This shows that the respondents were unlikely to pose any non-carcinogenic health effects. A study by Obiri *et al.,* (2005) revealed that the results of the non-cancer human health risk towards to the exposure through water sources were also found in most cases which greater than the USEPA's acceptable non-cancer to human health hazard index of 1. The mean of Lifetime Average Daily Dose (LADD) for Kampung Sedohok was slightly higher compared to Kampung Punjut. LCR indicated for the health risk for carcinogenic health effects encountered by the respondents. The results show that all of the respondents for both villages fall under the clearly acceptable risk in which the LCR value was less than 1×10^{-6}. This means that an additional one case of cancer was accepted for populations of 100, 000 or 1 000, 000, respectively. A risk level of 1 in a million, or 1 in one hundred thousand also implies that a likelihood that up to one person out of one million (or 100,000) was equally exposed people which would contract cancer if exposed continuously (24 hours per day) to a specific exposure dose over 70 years which an assume of average lifetime (USEPA, 2009). Most of the respondents in both villages fall under the clearly acceptable risk might due to the low level of arsenic concentration detected in the fresh water fish sample which unlikely to pose any health effects to the respondents. The mean concentration of arsenic in fresh water fish in both villages were below the permitted level. Based on ADD and LADD value of the respondents for both villages, the mean were not exceeded Provisional Tolerable Weekly Intake (PTWI) for arsenic which is 0.015 mg /kg.

5.6 The comparison of arsenic concentration in freshwater fish between the two areas

The comparison of arsenic concentration in fresh water fish between the two areas was analyzed using Mann-Whitney U test in order to determine whether there is a significant difference between the arsenic concentrations in freshwater fish of the two villages. Based on the results obtained there was no significant difference between the arsenic concentration in freshwater fish between the two villages. There were fewer studies related to the comparison of arsenic concentration in freshwater especially between two areas. However, a study by Kamarruzaman *et al.,* (2011) related to heavy metal which includes arsenic in South West Malaysian Coast showed the arsenic concentration was below the maximum permitted level. This might due to the fewer samples of fish to compare between the two areas. Based on this study, the fish samples were only 6 samples in which 3 each for each village. Furthermore, the mean concentration of the arsenic level in the freshwater fish samples for both villages was below the acceptable limit for permitted level of Malaysian Food Regulation 1985. So, it does not pose any significant difference of the arsenic concentration in the freshwater fish between the two villages. Even though, the level of arsenic in both villages were below the standard, it might be a problem if the exposure of arsenic continues for a long term and the level might increase especially in the aquatic organism.

5.7 The comparison of health risk assessment of the respondents between the two areas

The comparison of health risk assessment of the respondents between the two villages show that the there was a significant difference between the health risk of Hazard Quotient (HQ) and Lifetime Cancer Risk (LCR) among the respondents in the two villages. It showed that the mean intake of freshwater fish in Kampung Sedohok was significantly higher compared to Kampung Punjut. It also showed that the mean arsenic level detected in freshwater fish sample was significantly higher compared to Kampung Punjut. Hence, this explained that the higher significant of the mean intake of freshwater fish that detected with higher arsenic level in Kampung Sedohok correspond to the health risk faced by the respondents as compared to the respondents in Kampung Punjut. A study done by Qaiyum *et al.,* (2011) related to the difference sources of pollution condition in two different villages in Batu Pahat, Johor showed that there was a significant difference between health risks faced by respondents in the two villages. This was due to the difference pollution level of both villages. Hence, this explained that the pollution level of arsenic in both villages and ingestion rate of freshwater fish will affect the health risk of respondents.

Arsenic had also classified as carcinogenic to human. Based on the result, the Lifetime Cancer Risk (LCR) showed that respondents in Kampung Sedohok posed significantly higher cancer risk as compared to Kampung Punjut. The the risk value of LCR for Kampung Sedohok was 1.72×10^{-8}. This risk value indicated that freshwater fish consumption would result to an excess of 2 cancer cases per 100,000,000 populations. While for the respondents in Kampung Punjut, the risk value of LCR was 0.36×10^{-8}. This indicated an excess of 1 cancer case per 100,000,000 populations. This explained through a study by Victoria *et al.* (2011) which stated that the average Lifetime Cancer Risk (LCR) associated with the fish consumption for each species which considering the mean arsenic levels in the sampling locations.

5.8 The relationship between the fresh water fish frequency intake and health risk assessment of the respondents

A significant positive relationship was shown between the freshwater fish frequency intake and the health risk of HQ and LCR of the respondents in Kampung Sedohok with r = 0.790 (HQ); 0.787 (LCR). There were no respondents who exceed the acceptable limit value. This means that all the respondents were in a safe level. However, there was no significant relationship was shown between the freshwater fish frequency intake and the health risk of HQ and LCR of the respondents in Kampung Punjut. The frequency of freshwater fish intake influenced the data of health risk which were HQ and LCR. Based on WHO (2010), the intake of small quantities of organic arsenic through the fish organism does not pose a health risk. Based on the findings, most of the respondents in Kampung Sedohok were frequently consumed freshwater fish with a slightly higher mean intake freshwater fish of the respondents compared to Kampung Punjut. This indicated that frequent exposure to food contaminated with arsenic will increase the health risk. Furthermore, the value of correlation coefficient, r for both Hazard Quotient (HQ) and Lifetime Cancer Risk (LCR) for Kampung Sedohok show a greater relationship with the fresh water fish frequency intake. Based on the Guildfold rule of thumb, it shows that the value of correlation coefficient, r for both Hazard Quotient (HQ) and Lifetime Cancer Risk (LCR) for Kampung Sedohok in the ranged of 0.7- 0.9. Hence, this showed that the there was a strong relationship of freshwater fish frequency intake with the health risk of respondents in Kampung Sedohok. It can be explained that the increase in the freshwater fish intake that contaminated with arsenic will correspond with the increasing of the health risk faced among the respondents. Frequent consumption of freshwater fish that contaminated with arsenic may increase the As accumulation in the body in which later can cause detrimental effect to several health impacts. Marti *et al.,* (2008) indicated that fish and shellfish are the main groups that showing the highest contribution to the dietary intake of inorganic arsenic. Essentially, fish had the characteristic to metabolize, concentrate and store water borne pollutants such as arsenic (EFSA., 2009). Due to the characteristic of fish, it can harm people through the food chain in which the contaminated fish with arsenic are consumed by human through their diet.

The strength of this study was in term of methodology which for the detection of arsenic was using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). ICP-MS was also known as an analytical technique that performs elemental analysis with excellent sensitivity. The typical detection limit for ICP-MS was 0.01 to 1 microgram per liter in solution. ICP-MS can perform very fast whereby it can detect up to 60 elements in a 2 minutes scan. Furthermore, this study able to get the data and fish sample from the polluted river of Kluang, Johor. The limitation of this study research was lack of biological sample in which to assess the actual burden of arsenic in body of respondents. Furthermore, this study also has smaller sample size due to the less number of respondents from each village that matched with the inclusive criteria. The number of fish samples was less due the budget of analysis using ICP-MS. But, this study shows a strong potential for future research due to detection of contamination level of arsenic in the freshwater fish.

CONCLUSION

In conclusion, arsenic concentration in freshwater fish in polluted river of Sembrong show slightly higher as compared with less polluted river of Kahang. The mean concentration of arsenic in freshwater fish for both villages was below the maximum permitted level (1 mg/kg) by Malaysian Food Regulation 1985. There was no significant difference of the arsenic concentration in freshwater fish between Kampung Sedohok and Kampung Punjut. The health risk assessment for Hazard Quotient (HQ) and Lifetime Cancer Risk (LCR) of the respondents for both villages were still in acceptable level. However, there was a significant difference between the health risk faced by respondents between the both villages. It showed that the health risk faced by respondents in Kampung Sedohok significantly higher compared to Kampung Punjut. Furthermore, there was a significant relationship of freshwater fish frequency intake and the health risk for HQ and LCR of the respondents in Kampung Sedohok compared to Kampung Punjut.

RECOMMENDATION

1. Based on this study, some recommendation and improvements should be done in term of the methodology and study design in order to improve the study for better findings. In order to improve the methodology, actual health risk encountered by the respondent should be accessed through taking biological samples. Biological monitoring should be conducted in order to confirm the arsenic burden in human body of the respondents. For the purposes of this study for future improvements, blood and urine samples can be taken and analyzed to detect the arsenic level in the human body. This can determine the accumulation of the arsenic in the respondents through the consumption of the freshwater fish that contaminated with arsenic.
2. Other than that, few nutritional intake had been proved can detoxify the arsenic accumulation in human body. Communities are advocated to consume sulphur rich foods such as garlic, onions, legumes, poultry and eggs. It was found that sulphur can protects the cell from effects of toxin and it help in the formation of bile. Furthermore, an increase in fiber rich food intake could also good for health of the community. A study by Jeremy and Kaslar., (2011) stated that through the consumption of vegetables and fruits provide sources of fiber.
3. Environmental samples such as water and sediment sample can be taken and analyzed in order to confirm the arsenic concentration in water. Generally,

arsenic in water is inorganic form and the most toxic compared to the freshwater and marine organism.

REFERENCE

Agency for Toxic Substance and Disease Registry (ATSDR). (2007). Toxicological Profile for Arsenic. US Department of Health and Humans Services, Public Health Human Services, Centers for Diseases Control, Atlanta.

Ahmad, A.K , Suhaimi O.M and Ali M.M. (2009). A temporal study of selected metals concentration in fishes of Lake Chini, Peninsular Malaysia. *Malaysian Journal Analytical Science*, 13: 100-106.

Alina, M., Azrina, A., Moh Yunus, A.S., Mohd Zakiuddin, S., Mohd Izuan Effendi., and Muhammad Rizal, R. (2012). Heavy metals (mercury, arsenic, cadmium, plumbum) in selected marine fish and shellfish along the Straits of Malacca. *International Food Research Journal*, 19(1): 135-140.

Attar, M.A., El-Faer, M.Z., Rawadah, T.N., and Twabim, B. S. (1992). Levels of arsenic in fish from the Arabean Gulf. *Marine Pollution Bulletin*, 24: 94-97

Aziemah Z., Saliza, M.E., and Ahmad Z.A. (2012). *Arsenic concentration in shrimp paste and health risk assessment in two villages of Melaka*. Department of Environmental and Occupational Health.

Bayen, S., Thomas, G. O., Hian, K. L., and Obbard, J. P. (2003). Organochlorine pesticides and heavy metals in green mussel, perna viridis in Singapore. *Water, Air, and Soil Pollution*, 155: 103–116.

Bernhard, J., Beaufrere, B., Laville, M., and Fouque, D. (2004). Adaptive response to a low-protein diet in predialysis chronic renal failure patients. *Journal of the American Society of Nephrology,* 12:1249–1254.

Bernstam, L., and Nriagu, J. (2000). Molecular aspects of arsenic stress. *Journal of Toxicology and Environmental Health Part B*, 3:293-322.

Bhupander, K., Mukherjee, D. P., Sanjay, K., Meenu, M., Singh, K., and Sharma, C. S. (2011). Bioaccumlation of heavy metals in muscle tissue of fishes from selected aquaculture ponds in east Kolkata wetlands. *Analytical of Biological Research*, 2011, 2(5): 125-134.

Brooks, W.E. (2009). 2008 Minerals Yearbook: Arsenic. U.S. Geological Survey. http://minerals.usgs.gov/minerals/pubs/commodity/arsenic/myb1-2008-arsen.pdf. Date of retrieved: 06 May 2013.

Budiati, T. (2010). The presence of arsenic as heavy metal contaminant on salmon: a risk assessment. *International Journal of Basic and Applied Sciences,* 10 (5): 6-12.

Carbonell-Barrachina, A.A., Signes-Pastor,A.J., Vazquez-Araffljo, L., Burlo, F., and Sengupta, B. (2009). Presence of arsenic in agricultural products from arsenic-endemic areas and strategies to reduce arsenic intake in rural villages. *Journal of Molecules Nutrition and Food Resources*, 53:531-541.

Castro-Gonzalez M.I. and Mendez-Armenta M. (2008). Heavy metals: implications associated to fish consumption. *Environmental Toxicology and Pharmacology*, 26: 263-271.

Chong, Y. H., Tee, E. S., and Ng, T.K.W. (1984). Status of community nutrition in poverty kampungs. *Bulletin Instirute for Medical Research*, Kuala Lumpur.

Clement, B., and Gary, C., (2004). *Contaminant Accumulation in the Distribution System, Case Histories.* In: Inorganic Contaminants Workshop, February 1-3,. AWWA: Reno, Nevada.

Das, S., Unni, B., Bhattacharjee, M., Wann, S.B., & Rao, P.G. (2011). Toxicological effects of arsenic exposure in a freshwater teleost fish, Channa punctatus. *African Journal of Biotechnology*, 11(19): 4447-4454.

Department of Environment, (2010). "Environmental Quality Report- Technical Report, Department of Environment, Kuala Lumpur, Malaysia.

Domingo, J.L, Bocio, A. Falco, G and Llobet, J. M. (2007). Benefits and risks of fish consumption Part I. A quantitative analysis of the intake of omega-3 fatty acids and chemical contaminants. *Journal of Toxicology*, 230: 219-226.

Donohue, J.M. and Abernathy C.O. (2002). *Exposure to Inorganic Arsenic (As) from fish and Shellfish*. Office of Science and Technology, USEPA, Washington, D.C.

Eisler, R. (1988). *Arsenic hazards to fish, wildlife and invertebrates: A synoptic Review*. U.S Fish and Wildlife Service, Patuxent Wildlife Research Center, Laurel, MD, USA. 59-67

Environmental Protection Agency (EPA). (2000). Sediment quality guidelines developed for the national status and trends program. http://www.epa.gov/R5Super/ecology/benchmemo.htm. Date of retrieved: 24 March 2013.

European Food Safety Authority. (2009). Opinion of the scientific panel on contaminants in the food chain on a request from the European parliament related to the safety assessment of wild and farmed fish. *European Food Safety Authority Journal,* 236: 1-118.

European Food Safety Authority (2009). Scientific opinion on arsenic in food. *European Food Safety Authority Journal,* 7(10):1351.

FAO – Joint FAO/WHO expert Committee on food additives (1989). *Evaluation of certain food additives and contaminants,* Technical Report - 33rd Report, World Health Organization, Geneva, Switzerland, : 27-38.

Fathi Alhashmi Bashir., Mohammad Shuhaimi-Othman, and Mazlan, A.G. (2012). Evaluation of trace metal levels in Tissues of Two Commercial fish species in Kapar and Mersing Coastal waters, Peninsular Malaysia. *Journal of Environmental and Public Health.* 2012 (2012):10 .

Hajeb P., Jinap S., Ismail A., Fatimah A.B., Jamilah B. And Abdul Rahim M. (2009). Assessment of mercury level in commonly consumed marine fishes in Malaysia. *Food Control*, 20(1): 79-84.

Han, B.C., Jeng, W.L., Chen, R.Y., and Fang, G.T. (1998). Estimation of target hazard quotients and potential health risks for metals by consumption of seafood in Taiwan. *Environmental Contamination Toxicology*, 35: 711-720.

Hasegawa, H., Sohrin, Y., Seki, K., Sato, M., Norisuye, K., Naito, K., and Matsui, M. (2001). Biosynthesis and release of methylarsenic compounds during the growth of freshwater algae. *Chemosphere*, 43: 265-272.

Horacio O., Gonazalez, Roling J.A., Baldwin W.S., and Bain L.J. (2006). Physiological changes and differential gene expression in mummichogs (Fundulus heteroclitus) exposed to arsenic. *Aquatic Toxicology*. 77: 43-52.

International Agency for Research on Cancer (IARC). (1994). Monographs on the Evaluation of Carcinogenic Risks to Humans. 60: 389.

International Programme on Chemical Safety (2001). Chemical Safety Information from Intergovernmental Organization – Arsenic. http://www.inchem.org. Date of retrieved: 26 April 2013.

Jeremy, E., and Kaslow, M. D. (2011). Physician and surgeon .http://www.drkaslow.com/html/arsenic.html. Date of retrieved: 06 May 2013.

Kamaruzzaman, B. Y., Ong, M. C., Zaleha, K. and Shahbudin, S. (2008). Levels of Heavy metals in Green-Lipped Mussel Perna veridis (Linnaeus) from Muar Estuary, Johore, Malaysia. *Pakistan Journal of Biological Sciences,* 11(18): 2249-2253.

Kapaj, S., Peterson, H., Liver, K., and Bhattacharya, P. (2006). Human health effects from chronic arsenic poisoning- A review. *Journal of Environmental Science and Health Part A,* 42: 2399-2428.

Liao, C.M., and Ling, M.P. (2003). Assessment of human health risks for arsenic bioaccumulation in tilapia (Oreochromis mossambicus) and large-scale mullet (Liza macrolepis) from blackfoot disease area in Taiwan. *Archives of Environmental Contamination and Toxicology.* 56(3): 264-272.

Liu, J., Goyer, R.A., and Waalker, M.P. (2005). *Toxic effects of metals*. Caserrett and Doull's Toxicology the Basic Science of Poisons.pp. 931-979, McGraw-Hill, New York, NY, USA.

Lu, A. A., and Llewellyn, J. C., (2006). Designing and Conducting Health Surveys: A Comprehensive Guide. John Wiley & Sons. Inc. San Francisco, CA.

Malaysian Food Act (1983). Food Act 1983 (Act 281) & Regulations (1985), International Law book Services, Kuala Lumpur. 307.

Malik A. (2004) "Metal bioremediation through growing cells," Environment International, Volume 30(2) :261-278.

Mallory, A.L (2010). A Risk Assessment for Ingestion of Toxic Chemical in Fish from Imperial Beach, California. San Diego. State University. 67-70

Marti, R., Perello,G., and Domingo, J.L., (2007). Dietary exposure to metals by individuals living near a hazardous waste incinerator in Catalonia, Spain: Temporal trend. *Biological Trace Element Research*, 131(3): 245-54.

Mason, R.P., Laporte, J.M., and Andres, S. (2000). Factors controlling the bioaccumulation of mercury, methylmercury, arsenic, selenium and cadmium by freshwater invertebrates and fish. *Archives of Environmental Contamination. Toxicology,* 38:283–297.

Norimah A.K.,, Safiah. M., Jamal K., Siti Haslinda., Zuhaida H., Rohida S., Fatimah S., Siti Norazlina., Poh B.K., Kandiah M., Zalilah M.S., Wan Manan W.M., Fatimah S., and Azmi M.Y (2008). Food Consumption Patterns: Findings from the Malaysian Adult Nutrition Survey (MANS). *Nutrition Malaysian Jurnal*, 14(1): 25-39.

Obiri, S., Dodoo, D.K., Okai-Sam, F., and Essumang, D.K. (2005). Non-cancer health risk assessment from exposure to cyanide by resident adults from the mining operations to Bogoso Gold Limited in Ghana. *Environmental Monitoring and Assessment,* 118: 51-63.

Paquin R.R, Farley K, and Santore R.C (2003). Metals in Aquactic Systems: A review of Exposure, Bioaccumulation, and Toxicity Models, Society of Environmental Toxicology and Chemistry, Pensacola, Fla, USA.

Qaiyum, M. S., Shaharudin, M. S., Syazwan, A. I., and Muhaimin. A. (2011). Health risk assessment after exposure to aluminium in drinking water between two different villages. *Journal of water resource and production*, 3: 268-274.

Rashed M.N, (2001). Egypt monitoring of environmental heavy metals in M.cryprinoids from the harbor waters of Visakhapatnam. *Bulletin of Pure Applied Science*, 19: 77- 85.

Ratraike, R. (2003). Acute and Chronic Arsenic toxicity. *Postgraduate Medicine Journal*, 79 (933):391-396.

Rosemond, S. D., Xie, Q., and Liber, K. (2008). Arsenic concentration and speciation in five freshwater fish species from Back Bay near YellowKnife, NT, Canada. *Environment Monitoring Assess*, 149:199-210.

Safiuddin, M., Shirazi, S.M., and Yusoff, S. (2011). Arsenic contamination of groundwater in Bangladesh: A review. *International Journal of the Physical Sciences.* 6 (30): 6791 – 6800.

Sangeeta, D., Balagopalan, U., Minakshi, B., Sawlang, B. W., and Paruchuri, G.R. (2011). Toxicological effects of arsenic exposure in a freshwater teleost fish, Channa punctatus. *African Journal of Biotechnology*, 11(19): 4447-4454.

Sarkar, D., and Datta, R. (2004). Arsenic fate and bioavailability in two soils contaminated with sodium arsenate pesticide: An incubation study. *Bulletin Environmental Contamination and Toxicology,* 72: 240-247.

Saxe, J. K., Bowers, T. S., and Reid, K. R. (2006). Arsenic. In Environmental Forensics Contaminant Specific Guide (R. D. Morrison and B. L. Murphy, Eds. Academic Press, Burlington, MA.279–292.

Shariff, R., Ghazali, A.R., Rajab, N. F., Haron, H., and Osman, F. (2008). Toxicological Evaluation of some Malaysian locally processed raw food products. *Food and Chemical Toxicology,* 46: 368-364.

Shaw J., Colbourne J., Davey J., Glaholt S., Hampton T., Chen C., Folt C., and Hamilton J. (2007). The influence of treatment history on arsenic accumulation and toxicity in the killifish, Fundulus heteroclitus. *Environmental Toxicology Chemistry.* 26(12): 2704-2709.

Shinichi, M., Masahito, S., Yoshiaki, K., Takayoshi, K., Osamu, S., Shoko, F., Kevin, A. F., and Toshikazu, K. (2009). Rapid determination of arsenic species in freshwater organisms from the arsenic-rich Hayakawa River in Japan using HPLC-ICP-MS. *Chemosphere,* 75: 1065-1073.

Slejkovec, Z., Bajc, Z., and Doganoc, D. Z. (2004). Arsenic speciation patterns in freshwater fish. *Talanta,* 62: 931-936.

Smedley, P.L., and Kinniburgh, D.G. (2002). A review of the source, behaviour and distribution of arsenic in natural waters. *Applied Geochemistry,* 17:517-568.

Suzilla, Y., Saliza, M. E., and Ahmad, Z. A. (2012). Arsenic concentration in Anchovy sauce and health risk assessment among residents in Tumpat, Kelantan. Department of Environmental and Occupational Health.

Tucker, K.L., Bianchi, L.A., and Maras, J. (2007). Adaptation of a food frequency questionnaire to assess diets of Puerto Rican and non-Hispanic adults. *American Journal of Epidemiology,* 148 (5):507-518.

Tukiman, L., Norazura, I., Ahmad, M., and Sahibin, A. R. (2006). Kandungan logam berat dalam makanan laut dan kadar pengambilannya oleh penduduk di Tanjung Karang, Selangor. *The Malaysian Journal of Analytical Sciences*, 10 (2): 197–204.

Tuzen, M. (2003). Determination of heavy metals in fish samples of the middle Black Sea (Turkey) by graphite furnace atomic absorption spectrometry. *Food Chemistry,* 80(1):119 – 123.

US EPA (US Environmental Protection Agency) (2007). *Guidance manual for assessing human health risks from chemically contaminated, fish and shellfish*. EPA-503/8-89-002, US EPA Office of Marine and Estuarine Protection,Washington, DC.

US FDA (US Food and Drug Administration) (1993) Guidance document for arsenic in shellfish. US Food and Drug Administration,Washington, DC, 25–27.

Van Hulle, M., Zhang, C., Schotte, B., Mees, L., Vanhaecke, F., Vanholder, R., Zhang, X.R., and Cornelis, R. (2004). Identification of some arsenic species in human urine and blood after ingestion of Chinese seaweed Laminaric. *Journal Analytical Atomic Spectrometer,* 19 (1): 58-64.

Victoria B. M. (2011). Health Risk Assessment of Heavy Metals Bioaccumulation in Laguna de Bay Fish Products. Department of Environmental and Occupational Health. University of the Phillippines Manila.

Wan Abdul Manan W.M.,, Nur Firdaus I., Safiah M.Y., Siti Haslinda M.D., Poh, B.K., Norimah A.K., Azmi M.Y., Tahir A., Mimalini K., Zalilah M.S., Fatimah S., Siti Norazlin M.N., and Fasiah W. (2012). Meal pattern of Malaysian Adults:Finding from the Malaysian Adults Nutrition Survey (MANS). *Malaysian Nutrition Jurnal,* 18(2): 221-230.

Wang, L.X., Guo, Z. H., Xiao, X.Y., Chen, T. B., Liao, X. Y., and Song, J. (2008). Heavy metal pollution of soils and vegetables in the midstream and downstream of the Xiangjiang River, Human Province. *Journal of Geographical Sciences,* 18(3): 353-362.

World Health Organization, (1981). *Task group on Environmental Health criteria for arsenic: arsenic*. Environmental Health criteria 18, WHO Geneva, Switzerland: 1-174.

Zheng, J., and Hintelmann, H. (2004). Hyphenation of high performance liquid chromatography with sector field Inductively Coupled Plasma Mass Spectrometry for the determination of ultra-trace level anionic and cationic arsenic compounds in freshwater fish. *Journal of Analytical Atomic Spectrometery,* 19: 191-195.

Zukowska J., and Bizuk M, (2008). Methodological Evaluation of Method for Dietary Heavy metal intake, *Journal of food science,* Institute of Food Technologists,Gdansk: Poland.

Zuraini, A., Somchit., Solihah, M.H., Goh, Y.M., Arifah, A.K, Zakana, M.S., Somchit, N., Rallon, M.A., Zakaria, Z.A, and Mat Jais, A.M (2006). Fatty acid and amino acid composition of three local Malaysian channa spp. fish. *Food Chemistry,* 97(4): 674-678.

APPENDICE 1
UNIVERSITI RESEARCH ETHICS COMMITTEE (JKEUPM) APPROVAL LETTER

APPENDICE 2

JABATAN KEMAJUAN ORANG ASLI MALAYSIA (JAKOA) APPROVAL LETTER

JABATAN KEMAJUAN ORANG ASLI MALAYSIA
(KEMENTERIAN KEMAJUAN LUAR BANDAR DAN WILAYAH)
TINGKAT 10, 20 & 20M, WEST BLOCK,
WISMA SELANGOR DREDGING,
142 – C, JALAN AMPANG,
50548 KUALA LUMPUR.

JAKOA
JABATAN KEMAJUAN ORANG ASLI

Telefon : 03-21610577
: 03-21610994-8(5 talian)
Gombak : 03-61892122
Fax : 03-21621470 (IP)
: 03-61883160 (GBK)
Laman Web : www.jakoa.gov.my

65

Ruj. Kami : JAKOA.PP.30.032 Jld.26 (22)
Tarikh : 27 R' Awal 1434H
8 Februari 2013

PEJABAT KEMAJUAN ORANG ASLI DAERAH KLUANG
DITERIMA
19 FEB 2013

Timbalan Dekan
(Akademik: Sains Kesihatan)
Fakulti Perubatan dan Sains Kesihatan
Universiti Putra Malaysia
43400 UPM Serdang, Selangor
(u/p: Prof. Dr. Zalilah Mohd. Shariff)

Tuan,

KEBENARAN MENJALANKAN KAJIAN/PENYELIDIKAN

Dengan hormatnya saya diarah merujuk kepada perkara di atas.

2. Sukacita dimaklumkan bahawa Jabatan ini tiada halangan untuk memberi kebenaran kepada pelajar tuan untuk menjalankan kajian bertajuk **"Arsenic Concentration in Freshwater Fish and Health Risk Assessment Among Adults in Two Area at Kluang, Johor"**. Pihak tuan dibenarkan menjalankan kajian di tempat dan pada tarikh yang telah ditetapkan seperti berikut:-

Tempat	:	**Kg. Sedohok, Kg. Puncur, Sembrong, Kluang, Johor**
Tarikh	:	**8 Februari – 7 Mac 2013**
Penyelidik	:	**Christopher George**

3. Pihak tuan adalah diminta supaya dapat mematuhi syarat-syarat seperti mana terkandung dalam borang permohonan seperti di lampiran 'Appendix 1'. Di samping itu, pihak tuan juga diminta mengemukakan 2 salinan *hard copy* dan 1 salinan *soft copy* kepada JAKOA Ibu Pejabat ***(Bahagian Perancangan dan Penyelidikan)***.

PEJ. KEMAJUAN ORANG ASLI DAERAH KLUANG
TANDATANGAN:

PEGAWAI KOA		
PEMBANGUNAN SOSIOEKONOMI	TINDAKAN	
PERANCANGAN & PENYELIDIKAN	MAKLUMAT	
KESIHATAN	EDARAN	
MINDA & PENDIDIKAN		

APPENDICE 3
INFORMATION SHEET

JAWATANKUASA ETIKA UNIVERSITI UNTUK PENYELIDIKAN MELIBATKAN MANUSIA (JKEUPM)
UNIVERSITI PUTRA MALAYSIA, 43400 UPM SERDANG, SELANGOR, MALAYSIA

RESPONDENT'S INFORMATION SHEET

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

STUDY TITLE

ARSENIC CONCENTRATION IN FRESHWATER FISH AND HEALTH RISK ASSESSMENT AMONG ADULTS IN TWO AREAS AT KLUANG, JOHOR

INTRODUCTION

Heavy metal contamination in food sources are more concern among the communities because of the effects to the human health. Arsenic can be found naturally in the environment and cannot be destroyed or degraded. The occurrences of all these heavy metals to the environment are from the rapid industrialization, agricultural and uncontrolled urbanization which directly pollute the river basin that will contaminate the aquatic organism. Aquatic organism such as fish is one of the main sources of food that rich source of vitamins, minerals and proteins. So the issues regarding the heavy metal contamination in the freshwater river is important because it can directly effects the human health through the consumption of the contaminated fish This study is to determine the heavy metal, arsenic concentration in the fresh water fish and health risk assessment among adults in two areas which are Kampung Sedohok and Kampung Pucur located at Kluang, Johor.

WHAT WILL YOU HAVE TO DO?

The respondent is require to answer a questionnaire. It is a self-administered questionnaire. The questionnaire consists of 5 parts which include socio-demographic details, health status of the respondents, anthropometry details, frequency of food intake information and others possible sources of arsenic exposure. Next, the respondent will need to be taken blood samples (5 ml) and the blood sampling will be do by a medical assistant officer .This person had experienced in blood sampling using venipuncture method. The respondent's blood will be use to determine the arsenic body burden of the respondent associated with the freshwater fish consumption.

WHO SHOULD NOT ENTER THE STUDY?

Respondents below 18 years old and above 59 years old. Next, respondents that do not consume freshwater fish and respondents from immunocompromised group (such as cancer patient, HIV/Aids, transplant etc.) and pregnancy woman are excludes from the study. Respondents that have the inclusive criteria only are allowed to participate in this study.

WHAT WILL BE THE BENEFITS OF THE STUDY:

(a) TO YOU AS THE SUBJECT?

Respondents will be able to know about their health status and if there is a problem regarding to their health, the researcher will inform them immediately. The researcher will provide a free councelling session in order to maintain and improve their healthy life style. Moreover, information from this sudy may benefit other people now or in future. Information learned from this study will probably benefits other in the future.

b) TO THE INVESTIGATOR?

The researcher will gain the result and information regarding to the research study. After this study completed, it can be reviewed by other researchers if published.

WHAT ARE THE POSSIBLE RISKS?

The risk of participating for the respondents in this study are quite mild as it is required to take blood sample from each respondents. However, the researcher has taken precaution steps to minimize the risks of this study. Firstly, the blood sampling does not involve or use any harmful chemicals or drugs that can cause of death. Besides, the blood sample will only be taken by the registered and established physicians and the respondents will be given a choice whether to be a volunteer for this study or refused.

Before the blood sampling, the medical assistant officer will ask the respondent whether the respondent has allergies, phobias or had ever fainted during previous injections or blood flows. Next, respondents may experience dizziness and body weakness after the blood sampling is taken. However, it will not remain for a period of time. Respondent will recover after resting and taking a nap.

WILL THE INFORMATION THAT YOU PROVIDE AND YOUR IDENTITY REMAIN CONFIDENTIAL?

The information and identity of individual in this study will be remain private and confidential. It will be used solely in this study. Respondent will be identified in the research records only by a code name or number

JAWATANKUASA ETIKA UNIVERSITI UNTUK PENYELIDIKAN MELIBATKAN MANUSIA (JKEUPM)
UNIVERSITI PUTRA MALAYSIA, 43400 UPM SERDANG, SELANGOR, MALAYSIA

HELAIAN PENERANGAN RESPONDEN

Sila baca maklumat berikut dengan teliti. Sekiranya anda mempunyai sebarang pertanyaan, sila kemukakan kepada penyelidik.

TAJUK KAJIAN

Kepekatan arsenik dalam ikan air tawar dan penilaian risiko kesihatan kalangan orang dewasa di dua kawasan di Kluang, Johor

PENGENALAN

Pencemaran logam dalam sumber makanan menjadi perhatian dan diutamakan oleh masyarakat masa kini. Ini kerana kesannya kepada sistem tubuh yang boleh menjejaskan kesihatan seseorang individu. Arsenik merupakan unsur semulajadi dalam kerak bumi dan ianya tidak boleh dimusnahkan. Pencemaran arsenik adalah melalui beberapa sumber iaitu industri perkilangan dan juga penggunaan baja kimia dalam sumber pertanian. Sumber-sumber ini akan mencemarkan air sungai dan secara langsung akan terdedah kepada hidupan air seperti ikan. Ikan merupakan salah satu sumber makanan yang tinggi kandungan vitamin, mineral dan juga proteinnya. Isu pencemaran arsenik terhadap sungai adalah penting kerana ianya akan memberikan kesan yang buruk terhadap manusia melalui sumber makanan seperti ikan. Kajian ini menkaji mengenai kandungan arsenik dalam ikan air tawar dan penilaian risiko kesihatan kalangan orang dewasa di dua kawasan iaitu Kampung Sedohok dan Kampung Pucur di Kluang, Johor.

APAKAH YANG PERLU ANDA LAKUKAN?

Responden perlu menjawab borang soal-selidik yang akan diberikan kepada responden. Borang soal-selidik tersebut merangkumi 5 bahagian iaitu keterangan diri, status kesihatan responden, anthropometri, kekerapan pengambilan makanan dan juga faktor-faktor pendedahan lain. Selain itu, penyelidik memerlukan sampel darah responden sebanyak 5 ml untuk kajian ini dan prosedur pengambilan darah ini akan dilakukan oleh Penolong Pegawai Perubatan yang mempunyai pengalaman dalam pengambilan darah menggunakan kaedah 'Venipuncture'. Darah responden diambil adalah untuk mengenalpasti kepekatan arsenik dalam darah.

SIAPA YANG TIDAK BOLEH MENYERTAI KAJIAN INI?

Individu yang berumur 18 tahun ke bawah dan 59 tahun ke atas. Selain itu, individu yang tidak memakan ikan air tawar. Tambahan lagi, individu yang menghidap penyakit kanser, HIV/AIDS dan ibu mengandung tidak akan terlibat dengan kajian ini.

JAWATANKUASA ETIKA UNIVERSITI UNTUK
PENYELIDIKAN MELIBATKAN MANUSIA (JKEUPM)
UNIVERSITI PUTRA MALAYSIA, 43400 UPM SERDANG,
SELANGOR, MALAYSIA

APAKAH FAEDAH MENYERTAI KAJIAN INI?

a) KEPADA ANDA SEBAGAI PENYERTA?

Responden akan dapat mengetahui status kesihatan dan jika terdapat masalah berkenaan dengan kesihatan responden, penyelidik akan segera memberitahu kepada responden. Penyelidik juga akan menyediakan sesi kaunseling mengenai kesihatan kepada responden supaya kesihatan responden lebih terjamin. Selain itu, maklumat yang di dapati dalam kajian ini boleh membantu orang lain di masa hadapan.

b) KEPADA PENYELIDIK?

Penyelidik akan mendapat hasil keputusan dan maklumat berkenaan dengan kajian ini. Selepas kajian ini tamat, kajian ini boleh menjadi panduan kepada penyelidik lain.

ADAKAH IA BERISIKO?

Risiko kepada responden dalam kajian ini adalah tidak berbahaya terutamanya semasa prosedur pemgambilan darah responden. Ini kerana individu yang mengambil sampel darah tersebut adalah pengamal perubatan yang bertauliah dan berpengalaman dalam prosedur pengambilan darah. Walau bagaimanapun, penyelidik akan memastikan keselamatan responden yang terlibat dalam kajian ini. Seterusnya, prosedur pengambilan darah ini tidak menggunakan sebarang bahan kimia yang merbahaya. Responden juga berhak untuk menolak supaya tidak terlibat dengan kajian ini.

Sebelum prosedur pengambilan darah dijalankan, penolong pegawai perubatan tersebut akan bertanyakan kepada responden beberapa soalan mengenai kesihatan responden. Tambahan lagi, responden akan merasa pening selepas pengambilan darah. Responden akan kembali pulih selepas berehat atau tidur sementara.

ADAKAH MAKLUMAT DAN IDENTITI SAYA KEKAL RAHSIA?

Semua maklumat dan identiti individu-individu yang terlibat dalam kajian ini akan dirahsiakan dan sulit. Maklumat-maklumat tersebut cuma akan digunakan semasa kajian ini dan responden direkodkan berdasarkan nombor kod.

APPENDICE 4
RESPONDENT SHEET

CONSENT FORM (RESPONDENT)

RESEARCH TITLE : ARSENIC CONCENTRATION IN FRESHWATER FISH AND HEALTH RISK ASSESSMENT AMONG ADULTS IN TWO AREAS AT KLUANG, JOHOR.

RESEARCHER : CHRISTOPHER GEORGE

I .... Identity Card No. .... address.. ....hereby voluntarily agree to take part in the clinical research *(clinical study, questionnaire study/ drug trial) specified above.

I have been informed about the nature of the clinical research in terms of methodology, possible adverse effects and complications (refer to Information Sheet). I understand that I have the right to withdraw from this clinical research at any time without assigning any reason whatsoever. I also understand that this study is confidential and all information provided with regards to my identity will remain private and confidential.

I wish to *know/don't wish to know the results of the tests performed on my sample.

* delete where necessary

Signature .... (*Respondent*)

Date :..

Signature .... (*Witness*)

Name :..

I/C No. :..

I confirm that I have explained to the respondent the nature and purpose of the above – mentioned clinical research.

Date ....

Signature .... (Researcher)

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

BORANG PERSETUJUAN (RESPONDEN)

TAJUK KAJIAN :KEPEKATAN ARSENIK DALAM IKAN AIR TAWAR DAN PENILAIAN RISIKO KESIHATAN DALAM KALANGAN ORANG DEWASA DI KLUANG, JOHOR.

PENYELIDIK :CHRISTOPHER GEORGE

Saya .... No. Kad Pengenalan .... alamat .... dengan ini secara sukarela bersetuju untuk mengambil bahagian dalam penyelidikan klinikal * (kajian klinikal, soal selidik / dadah percubaan) yang dinyatakan di atas.

Saya telah dimaklumkan tentang sifat penyelidikan klinikal dari segi metodologi, kemungkinan kesan komplikasi dan buruk(rujuk kepada Lembaran Maklumat). Saya memahami bahawa saya mempunyai hak untuk menarik diri daripada penyelidikan klinikal ini pada bila-bila masa tanpa memberikan sebarang alasan. Saya juga memahami bahawa kajian ini adalah sulit dan semua maklumat yang diberikan berhubung identiti saya akan kekal sulit dan persendirian.

Saya * ingin / tidak ingin tahu keputusan ujian yang dilakukan ke atas sampel saya.

* Potong mana yang perlu

Tandatangan .... (*Responden*)

Tandatangan .... (*Saksi*)

Tarikh :..

Nama :..

No. KadPengenalan :..

Saya mengesahkan bahawa saya telah menerangkan kepada responden sifat dan tujuan penyelidikan klinikal tersebut di atas.

Tarikh ....

Tandatangan .... (Penyelidik)

APPENDICE 5
QUESTIONNAIRE

ID Peserta :

UPM UNIVERSITI PUTRA MALAYSIA BERILMU BERBAKTI	**JABATAN KESIHATAN PERSEKITARAN DAN PEKERJAAN FAKULTI PERUBATAN DAN SAINS KESIHATAN UNIVERSITI PUTRA MALAYSIA 43400, UPM SERDANG SELANGOR DARUL EHSAN**
	BORANG SOAL SELIDIK KAJIAN ILMIAH TAHUN AKHIR (EOH 4999)

TAJUK KAJIAN:

KANDUNGAN LOGAM BERAT DALAM IKAN DAN UDANG AIR TAWAR DAN PENILAIAN RISIKO KESIHATAN DI KALANGAN ORANG DEWASA DI KLUANG, JOHOR

PERHATIAN:

- Soal selidik ini mengandungi 5 bahagian utama: **Bahagian 1, 2, 3, 4 dan 5.**
- Responden diminta untuk menjawab **semua** soalan.
- Semua maklumat yang diberikan oleh responden akan dirahsiakan dan akan digunakan untuk tujuan kajian ini sahaja.
- Kerjasama daripada responden sepanjang kajian ini adalah amat dihargai.
- Terima kasih atas kerjasama dan masa anda.

FAKULTI PERUBATAN DAN SAINS KESIHATAN
UNIVERSITI PUTRA MALAYSIA, 43400 UPM SERDANG,
SELANGOR DARUL EHSAN, MALAYSIA

ID peserta: | | | |

BAHAGIAN 1	KETERANGAN DIRI

Sila isi dan pilih jawapan yang tepat.

1. Umur: ……………. Tahun

2. Jantina: a. Lelaki b. Perempuan

3. Bangsa: a. Melayu b. Cina c. India d. Lain-lain (Sila nyatakan): ....

4. Agama:
   a. Islam
   b. Kristian
   c. Buddha
   d. Hindu
   e. Lain-lain

   (Sila nyatakan): ……………......................

5. Status:
   a. Bujang
   b. Berkahwin
   c. Bercerai/berpisah
   d. Balu/Duda

6. Taraf pendidikan:
   a. Tidak Bersekolah
   b. Pra-Sekolah
   c. Sekolah Rendah
   d. Sekolah Menengah
   e. Sijil/STPM/Matrikulasi
   f. Ijazah Sarjana Muda
   g. Doktor Falsafah

7. Jenis pekerjaan:
   a. Kerajaan
   b. Swasta
   c. Bekerja Sendiri/ Suri rumah
   d. Pencen/Tidak Bekerja
   e. Lain-lain

8. Pendapatan sebulan (RM) : ______________

9. Pendapatan isi rumah sebulan (RM) : ______________

10. Bilangan ahli isi rumah : __________ orang

ID peserta: | | | |

BAHAGIAN 2	MAKLUMAT KESIHATAN

11. Adakah doktor mengesahkan anda mengalami masalah kesihatan berikut :	Ya	Tidak
a. Adakah anda mengalami kesukaran ketika membuang air kecil ?		
b. Adakah anda mengalami kerapuhan tulang?		
c. Adakah anda menghidap penyakit buah pinggang?		
d. Adakah anda mengalami sakit-sakit sendi?		
e. Adakah anda menghidap penyakit tulang?		
f. Adakah anda menghidap penyakit kulit? Cth : "Ezcema", gatal2, Kemerahan		
g. Adakah anda menghidap keradangan paru-paru?		
h. Adakah anda mengalami kesukaran untuk bernafas?		
i. Adakah anda menghidap kanser paru-paru?		
j. Adakah anda menpunyai masalah anemia (kekurangan darah merah) ?		
k. Adakah anda mengalami penyakit tekanan darah tinggi?		
l. Adakah anda menghidap penyakit gout?		
m. Adakah anda menghidap penyakit kanser?		

12. Adakah anda pernah mengalami simptom-simptom seperti berikut :	Ya	Tidak
a. Adakah anda pernah mengalami simptom muntah-muntah?		
b. Adakah anda pernah mengalami pening kepala?		
c. Adakah anda pernah mengalami kelesuan?		
d. Adakah anda pernah mengalami alahan pada kulit?		
e. Adakah anda mengalami asma?		
f. Adakah anda pernah mengalami kemurungan?		
g. Adakah anda pernah hilang selera makan?		
h. Adakah anda pernah kekejangan otot?		
i. Adakah anda pernah sakit perut/cirit-birit?		

13. Adakah anda telah mendapatkan rawatan untuk simptom-simptom di atas?

a. Ya, sila nyatakan bila kali terakhir anda mendapatkan rawatan: ……………………………………………………………… b. Tidak

ID peserta: | | | |

BAHAGIAN 3:	ANTHROPOMETRI
1. Berat (kg) :	i. [ ][ ][ ] . [ ] kg ii. [ ][ ][ ] . [ ] kg iii. [ ][ ][ ] . [ ] kg
2. Tinggi (cm) :	i. [ ][ ][ ] . [ ] cm ii. [ ][ ][ ] . [ ] cm iii. [ ][ ][ ] . [ ] 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

FAKULTI PERUBATAN DAN SAINS KESIHATAN
UNIVERSITI PUTRA MALAYSIA, 43400 UPM SERDANG,
SELANGOR DARUL EHSAN, MALAYSIA

ID peserta: | | | |

BAHAGIAN 4: BORANG KEKERAPAN PENGAMBILAN MAKANAN

	Jenis makanan	Kekerapan pengambilan					Ukuran sajian(pilih satu jenis ukuran sahaja)	Berapa banyak sajian setiap kali makan
	(A) Makanan Berunsurkan protein	Berapa kali sehari	Berapa kali seminggu	Berapa kali sebulan	Berapa kali setahun	Tidak makan		
A1	Ikan bilis						Sudu makan	
A2	Ikan dalam tin						Ekor	
A3	Kekerang						Sudu makan	
A4	Udang basah						Ekor sederhana	
A5	Sotong basah						Potong sederhana	
A6	Sotong kering						Keping sederhana	
							Potong sederhana	
A7	Ketam						Ekor	
A8	Ikan kering						Keping	
							Ekor	
A9	Bebola ikan/kek ikan						Bebola	
							Ketul	
A10	Kerepok lekor						Ketul	

Kod	Jenis makanan	Kekerapan pengambilan					Ukuran sajian(pilih satu jenis ukuran sahaja)	Berapa banyak sajian setiap kali makan
	(B)Kekacang dan hasilnya	Berapa kali sehari	Berapa kali seminggu	Berapa kali sebulan	Berapa kali setahun	Tidak makan		
B1	Kekacang						Sudu makan	
B2	Tauhu						Keping	
B3	Tempe						Keping	
							Sudu makan	
B4	Kacang Tanah						Sudu makan	

ID peserta: | | |

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		
C1	Sayuran berdaun						Cawan	
C2	Sayuran kacang						Cawan	
C3	Sayuran berubi						Cawan	
C4	Sayuran kobis						Cawan	
C5	Petola/labu /timun						Cawan	
C6	Ulam-ulaman						Cawan	
C7	Putik jagung						Sudu makan	
C8	Cendawan basah /kering						Cawan	
C9	Taugeh						Cawan	

Kod	Jenis makanan	Kekerapan pengambilan					Ukuran sajian(pilih satu jenis ukuran sahaja)	Berapa banyak sajian setiap kali makan
	(D)Makanan perencah /perasa	Berapa kali sehari	Berapa kali seminggu	Berapa kali sebulan	Berapa kali setahun	Tidak makan		
D1	Gula						Sudu teh	
D2	Madu						Sudu teh	
D3	Belacan						Sudu makan	
D4	Cencaluk						Sudu teh	
D5	Budu						Sudu teh	
D6	Kicap pekat						Sudu teh	
D7	Kicap cair						Sudu makan	
D8	Sos cili/tomato						Sudu makan	
D9	Sos tiram						Sudu teh	
D10	Sos ikan						Sudu teh	
D11	Otak udang						Sudu teh	

FAKULTI PERUBATAN DAN SAINS KESIHATAN
UNIVERSITI PUTRA MALAYSIA, 43400 UPM SERDANG,
SELANGOR DARUL EHSAN, MALAYSIA

Kod	Jenis makanan (E) 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
E1	Ikan air tawar (*Freshwater fish*)						Keping	
							Ekor	
E2	Ikan Baung (*Mystus nemurus*)						Keping	
							Ekor	
E3	Ikan Belida (*Notopterus Chitala)*						Keping	
							Ekor	
E4	Ikan Jelawat (*Leotobarbus hoeveni)*						Keping	
							Ekor	
E5	Ikan Kaloi (*Osphroremus Gorami*)						Keping	
							Ekor	
E6	Ikan Keli *(Catfish)*						Keping	
							Ikan	
E7	Ikan Patin *(Pangasius sp.)*						Keping	
							Ekor	
E8	Ikan Tilapia (*Tilapia hybrid*)						Keping	
							Ekor	
E9	Freshwater prawn /Udang Galah						Keping	
							Ekor	

Sumber :

☐ Pasar ☐ Tangkap sendiri

☐ Perusahaan kecil (IKS) ☐ Kolam/ Sangkar

FAKULTI PERUBATAN DAN SAINS KESIHATAN
UNIVERSITI PUTRA MALAYSIA, 43400 UPM SERDANG,
SELANGOR DARUL EHSAN, MALAYSIA

ID peserta: | | |

Senaraikan 3 spesis ikan/udang yang paling kerap anda makan?

No.	Jenis ikan/udang	Kekerapan pengambilan					Ukuran sajian(pilih satu jenis ukuran sahaja)	Berapa banyak sajian setiap kali makan
		Berapa kali sehari	Berapa kali seminggu	Berapa kali sebulan	Berapa kali setahun	Tidak makan		
1.							Keping	
							Ekor	
2.							Keping	
							Ekor	
3.							Keping	
							Ekor	
4.							Keping	
							Ekor	

Sejak bilakah anda mengamalkan pengambilan ikan air tawar/Udang sebagai salah satu menu harian?
Sila nyatakan : ______________________________tahun.

Bilakah kali terakhir anda memakan ikan air tawar/udang ? ____________________.

Sila nyatakan waktu pengambilan: ____________________________.

ID peserta: | | | |

BAHAGIAN 5:	FAKTOR-FAKTOR PENDEDAHAN LAIN

Sila pilih dan tandakan pada jawapan yang tepat.

a) Sumber bekalan air

1. Dari manakah anda mendapat sumber bekalan air minuman?

   a. Air sungai
   b. Air paip
   c. Air perigi
   d. Lain-lain : ________________

b) Pendedahan pekerjaan

2. Pernahkah pekerjaan anda melibatkan penggunaan pestisid (racun makhluk perosak)?
   a. Ya
      i. Sila nyatakan pekerjaan anda : _____________
      ii. Berapa lama anda terlibat dengan pekerjaan ini?
      ____
   b. Tidak

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

   a. Ya
   b. Tidak

c) Amalan gaya hidup (Merokok)

4. Adakah anda merokok?
   a. Ya
      i. Berapa tahun anda sudah merokok:
      Bil tahun: _____________

      ii. Nyatakan bilangan rokok yang dihisap dalam sehari:
      Bil. Batang rokok dalam sehari: _________

ID peserta: | | | |

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

a. Tidak sama sekali
b. Sedikit
c. Sederhana
d. Mendalam

b. Tidak

c. Sudah berhenti

i. Pada umur berapa anda berhenti merokok sepenuhnya?

____

d) Amalan gaya hidup (Pengambilan alcohol)

5. Adakah anda pernah mengambil minuman beralkohol?

a. Ya

i. Sila nyatakan berapa botol sehari anda minum? __________ botol

b. Tidak

6. Adakah anda pernah mengambil sebarang jenis ubat-ubatan?

a. Ya

i. Sila nyatakan jenis ubat-ubatan

____

ii. Nyatakan berapa kerap anda mengambilnya.

____

b. Tidak

7. Adakah anda pernah mengambil sebarang makanan tambahan?

a. Ya

i. Sila nyatakan jenis makanan tambahan.

____

ii. Nyatakan kekerapan anda mengambilnya.

____

b. Tidak

"TERIMA KASIH ATAS KERJASAMA ANDA"

APPENDICE 6

ICP - RESULTS

Quantitative Analysis - Summary Report

Sample ID: Blank
Sample Date/Time: Friday, May 03, 2013 09:27:36
Sample Description:
Solution Type: Blank
Blank File: C:\Elandata\DataSet\Dr Saliza (FPSK)\Blank.002
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\Sample\Dr Saliza (FPSK).sam
Method File: C:\Elandata\Method\dr saliza (fpsk).mth
Dataset File: C:\Elandata\DataSet\Dr Saliza (FPSK)\Blank.002
Tuning File: C:\Elandata\Tuning\default.tun
Optimization File: C:\Elandata\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	76	16.079		
Pb	208	1167	2.722		
Cr	52	12655	2.014		
Cd	111	21	3.685		

Concentration Results

Analyte	Mass	Net Intens. Mean	Conc. Mean	Conc. SD	Conc. RSD	Sample Unit
As	75					ppb
Pb	208					ppb
Cr	52					ppb
Cd	111					ppb

Replicates

Repeat 1

Analyte	Concentration
As	
Pb	
Cr	
Cd	

Repeat 2

Analyte	Concentration
As	
Pb	
Cr	
Cd	

Repeat 3

Analyte	Concentration
As	
Pb	
Cr	

Quantitative Analysis - Summary Report

Sample ID: Std 1 (5 ppb)
Sample Date/Time: Friday, May 03, 2013 09:29:14
Sample Description:
Solution Type: Standard
Blank File: C:\Elandata\DataSet\Dr Saliza (FPSK)\Blank.002
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\Sample\Dr Saliza (FPSK).sam
Method File: C:\Elandata\Method\dr saliza (fpsk).mth
Dataset File: C:\Elandata\DataSet\Dr Saliza (FPSK)\Std 1 (5 ppb).003
Tuning File: C:\Elandata\Tuning\default.tun
Optimization File: C:\Elandata\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	2396	2.925	76.000	16.079
Pb	208	108868	0.230	1166.742	2.722
Cr	52	64082	0.351	12655.027	2.014
Cd	111	21	9.750	20.889	3.685

Concentration Results

Analyte	Mass	Net Intens. Mean	Conc. Mean	Conc. SD	Conc. RSD	Sample Unit
As	75	2319.871	**5.000**	0.15	3.0	ppb
Pb	208	107701.704	**5.000**	0.01	0.2	ppb
Cr	52	51427.376	**5.000**	0.02	0.4	ppb
Cd	111	0.000	**5.000**	78111996.31	1562239924.7	ppb

Replicates

Repeat 1

Analyte	Concentration
As	4.825615
Pb	5.012320
Cr	4.977546
Cd	-85227168.578472

Repeat 2

Analyte	Concentration
As	5.090067
Pb	4.998476
Cr	5.001175
Cd	17045435.215694

Repeat 3

Analyte	Concentration
As	5.084318
Pb	4.989205
Cr	5.021279

Quantitative Analysis - Summary Report

Sample ID: Std 2 (10 ppb)

Sample Date/Time: Friday, May 03, 2013 09:30:52
Sample Description:
Solution Type: Standard
Blank File: C:\Elandata\DataSet\Dr Saliza (FPSK)\Blank.002
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\Sample\Dr Saliza (FPSK).sam
Method File: C:\Elandata\Method\dr saliza (fpsk).mth
Dataset File: C:\Elandata\DataSet\Dr Saliza (FPSK)\Std 2 (10 ppb).004
Tuning File: C:\Elandata\Tuning\default.tun
Optimization File: C:\Elandata\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	8566	2.736	76.000	16.079
Pb	208	201979	0.601	1166.742	2.722
Cr	52	114615	0.692	12655.027	2.014
Cd	111	26	28.005	20.889	3.685

Concentration Results

Analyte	Mass	Net Intens. Mean	Conc. Mean	Conc. SD	Conc. RSD	Sample Unit
As	75	8489.813	**10.997**	0.30	2.8	ppb
Pb	208	200812.416	**9.857**	0.06	0.6	ppb
Cr	52	101959.870	**9.982**	0.08	0.8	ppb
Cd	111	5.333	**12.500**	17.21	137.7	ppb

Replicates

Repeat 1

Analyte	Concentration
As	11.072942
Pb	9.800761
Cr	10.003273
Cd	-5.208329

Repeat 2

Analyte	Concentration
As	10.663223
Pb	9.919390
Cr	10.047560
Cd	13.541662

Repeat 3

Analyte	Concentration
As	11.256196
Pb	9.850135
Cr	9.896598

Quantitative Analysis - Summary Report

Sample ID: Std 3 (20 ppb)

Sample Date/Time: Friday, May 03, 2013 09:32:30
Sample Description:
Solution Type: Standard
Blank File: C:\Elandata\DataSet\Dr Saliza (FPSK)\Blank.002
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\Sample\Dr Saliza (FPSK).sam
Method File: C:\Elandata\Method\dr saliza (fpsk).mth
Dataset File: C:\Elandata\DataSet\Dr Saliza (FPSK)\Std 3 (20 ppb).005
Tuning File: C:\Elandata\Tuning\default.tun
Optimization File: C:\Elandata\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	20455	0.380	76.000	16.079
Pb	208	411033	0.951	1166.742	2.722
Cr	52	228532	3.120	12655.027	2.014
Cd	111	24	20.031	20.889	3.685

Concentration Results

Analyte	Mass	Net Intens. Mean	Conc. Mean	Conc. SD	Conc. RSD	Sample Unit
As	75	20378.541	**21.225**	0.08	0.4	ppb
Pb	208	409866.751	**20.028**	0.19	1.0	ppb
Cr	52	215877.378	**20.259**	0.67	3.3	ppb
Cd	111	3.111	**14.135**	21.84	154.5	ppb

Replicates

Repeat 1

Analyte	Concentration
As	21.253071
Pb	19.831805
Cr	19.687614
Cd	38.365381

Repeat 2

Analyte	Concentration
As	21.287866
Pb	20.213271
Cr	20.995173
Cd	8.076919

Repeat 3

Analyte	Concentration
As	21.133374
Pb	20.038884
Cr	20.094771

Quantitative Analysis - Summary Report

Sample ID: Std 4 (50 ppb)

Sample Date/Time: Friday, May 03, 2013 09:34:08
Sample Description:
Solution Type: Standard
Blank File: C:\Elandata\DataSet\Dr Saliza (FPSK)\Blank.002
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\Sample\Dr Saliza (FPSK).sam
Method File: C:\Elandata\Method\dr saliza (fpsk).mth
Dataset File: C:\Elandata\DataSet\Dr Saliza (FPSK)\Std 4 (50 ppb).006
Tuning File: C:\Elandata\Tuning\default.tun
Optimization File: C:\Elandata\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	56479	1.772	76.000	16.079
Pb	208	1049409	1.752	1166.742	2.722
Cr	52	566412	1.896	12655.027	2.014
Cd	111	41	5.587	20.889	3.685

Concentration Results

Analyte	Mass	Net Intens. Mean	Conc. Mean	Conc. SD	Conc. RSD	Sample Unit
As	75	56403.383	**51.326**	0.91	1.8	ppb
Pb	208	1048242.540	**50.208**	0.88	1.8	ppb
Cr	52	553756.980	**50.331**	0.98	1.9	ppb
Cd	111	20.445	**54.355**	6.14	11.3	ppb

Replicates

Repeat 1

Analyte	Concentration
As	50.533386
Pb	49.520649
Cr	49.824265
Cd	61.445325

Repeat 2

Analyte	Concentration
As	51.124247
Pb	49.902554
Cr	49.712107
Cd	50.810546

Repeat 3

Analyte	Concentration
As	52.320748
Pb	51.200440
Cr	51.455895

Quantitative Analysis - Summary Report

Sample ID: Std 5 (100 ppb)

Sample Date/Time: Friday, May 03, 2013 09:35:47
Sample Description:
Solution Type: Standard
Blank File: C:\Elandata\DataSet\Dr Saliza (FPSK)\Blank.002
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\Sample\Dr Saliza (FPSK).sam
Method File: C:\Elandata\Method\dr saliza (fpsk).mth
Dataset File: C:\Elandata\DataSet\Dr Saliza (FPSK)\Std 5 (100 ppb).007
Tuning File: C:\Elandata\Tuning\default.tun
Optimization File: C:\Elandata\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	115374	1.265	76.000	16.079
Pb	208	2053531	0.845	1166.742	2.722
Cr	52	1120434	3.306	12655.027	2.014
Cd	111	62	14.371	20.889	3.685

Concentration Results

Analyte	Mass	Net Intens. Mean	Conc. Mean	Conc. SD	Conc. RSD	Sample Unit
As	75	115298.243	**101.101**	1.28	1.3	ppb
Pb	208	2052364.175	**99.601**	0.84	0.8	ppb
Cr	52	1107779.324	**100.158**	3.35	3.3	ppb
Cd	111	40.889	**101.896**	22.12	21.7	ppb

Replicates

Repeat 1

Analyte	Concentration
As	99.697911
Pb	100.530145
Cr	97.056843
Cd	87.497864

Repeat 2

Analyte	Concentration
As	101.400440
Pb	99.384223
Cr	103.709770
Cd	90.820574

Repeat 3

Analyte	Concentration
As	102.204472
Pb	98.887299
Cr	99.708589

Quantitative Analysis - Summary Report

Sample ID: Std 6 (300 ppb)

Sample Date/Time: Friday, May 03, 2013 09:37:26
Sample Description:
Solution Type: Standard
Blank File: C:\Elandata\DataSet\Dr Saliza (FPSK)\Blank.002
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\Sample\Dr Saliza (FPSK).sam
Method File: C:\Elandata\Method\dr saliza (fpsk).mth
Dataset File: C:\Elandata\DataSet\Dr Saliza (FPSK)\Std 6 (300 ppb).008
Tuning File: C:\Elandata\Tuning\default.tun
Optimization File: C:\Elandata\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	340308	2.048	76.000	16.079
Pb	208	6142564	0.362	1166.742	2.722
Cr	52	3288184	2.011	12655.027	2.014
Cd	111	171	2.817	20.889	3.685

Concentration Results

Analyte	Mass	Net Intens. Mean	Conc. Mean	Conc. SD	Conc. RSD	Sample Unit
As	75	340232.418	**299.789**	6.14	2.0	ppb
Pb	208	6141397.014	**299.751**	1.08	0.4	ppb
Cr	52	3275529.168	**299.508**	6.05	2.0	ppb
Cd	111	149.779	**307.633**	9.87	3.2	ppb

Replicates

Repeat 1

Analyte	Concentration
As	297.876164
Pb	298.569661
Cr	295.385963
Cd	304.894289

Repeat 2

Analyte	Concentration
As	306.658504
Pb	300.700109
Cr	296.690145
Cd	299.417103

Repeat 3

Analyte	Concentration
As	294.831810
Pb	299.982568
Cr	306.448141

Calibration Report

Analyte	Mass	Curve Type	Slope	Intercept	Corr Coeff
As	74.922	Linear Thru Zero	1135.802136	0.000	0.999987
Pb	207.977	Linear Thru Zero	20488.343766	0.000	0.999992
Cr	51.941	Linear Thru Zero	10936.363163	0.000	0.999986
Cd	110.904	Linear Thru Zero	0.496861	0.000	0.999563

Quantitative Analysis - Summary Report

Sample ID: 1

Sample Date/Time: Friday, May 03, 2013 09:48:16
Sample Description: Fish 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\Sample\Dr Saliza (FPSK).sam
Method File: C:\Elandata\Method\dr saliza (fpsk).mth
Dataset File: C:\Elandata\DataSet\Dr Saliza (FPSK)\1.009
Tuning File: C:\Elandata\Tuning\default.tun
Optimization File: C:\Elandata\Optimize\default.dac
Calibration File: C:\Elandata\System\Dr Saliza (FPSK).cal
Calibration Type: External Calibration

Summary

Intensities

Analyte	Mass	Meas. Intens. Mean	Meas. Intens. RSD	Blank Intensity	Blank Intens. RSD
As	75	3876	3.189	76.000	16.079
Pb	208	307124	1.790	1166.742	2.722
Cr	52	473644	1.252	12655.027	2.014
Cd	111	4237	2.116	20.889	3.685

Concentration Results

Analyte	Mass	Net Intens. Mean	Conc. Mean	Conc. SD	Conc. RSD	Sample Unit
As	75	3799.937	**3.346**	0.11	3.3	ppb
Pb	208	305957.355	**14.933**	0.27	1.8	ppb
Cr	52	460988.854	**42.152**	0.54	1.3	ppb
Cd	111	4216.099	**8485.462**	180.42	2.1	ppb

Replicates

Repeat 1

Analyte	Concentration
As	3.430939
Pb	14.960980
Cr	42.776224
Cd	8657.286876

Repeat 2

Analyte	Concentration
As	3.382787
Pb	14.652117
Cr	41.883092
Cd	8297.528871

Repeat 3

Analyte	Concentration
As	3.223068
Pb	15.186623
Cr	41.796486

Quantitative Analysis - Summary Report

Sample ID: 2

Sample Date/Time: Friday, May 03, 2013 09:49:56
Sample Description: Fish 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\Sample\Dr Saliza (FPSK).sam
Method File: C:\Elandata\Method\dr saliza (fpsk).mth
Dataset File: C:\Elandata\DataSet\Dr Saliza (FPSK)\2.010
Tuning File: C:\Elandata\Tuning\default.tun
Optimization File: C:\Elandata\Optimize\default.dac
Calibration File: C:\Elandata\System\Dr Saliza (FPSK).cal
Calibration Type: External Calibration

Summary

Intensities

Analyte	Mass	Meas. Intens. Mean	Meas. Intens. RSD	Blank Intensity	Blank Intens. RSD
As	75	711	6.889	76.000	16.079
Pb	208	23937	2.559	1166.742	2.722
Cr	52	44043	1.851	12655.027	2.014
Cd	111	157	15.329	20.889	3.685

Concentration Results

Analyte	Mass	Net Intens. Mean	Conc. Mean	Conc. SD	Conc. RSD	Sample Unit
As	75	634.694	**0.559**	0.04	7.7	ppb
Pb	208	22770.077	**1.111**	0.03	2.7	ppb
Cr	52	31387.872	**2.870**	0.07	2.6	ppb
Cd	111	136.001	**273.721**	48.40	17.7	ppb

Replicates

Repeat 1

Analyte	Concentration
As	0.608115
Pb	1.103798
Cr	2.914598
Cd	223.627845

Repeat 2

Analyte	Concentration
As	0.528283
Pb	1.144319
Cr	2.784007
Cd	320.235919

Repeat 3

Analyte.	Concentration
As	0.540023
Pb	1.085986
Cr	2.911535

Quantitative Analysis - Summary Report

Sample ID: 3

Sample Date/Time: Friday, May 03, 2013 09:51:36
Sample Description: Fish 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\Sample\Dr Saliza (FPSK).sam
Method File: C:\Elandata\Method\dr saliza (fpsk).mth
Dataset File: C:\Elandata\DataSet\Dr Saliza (FPSK)\3.011
Tuning File: C:\Elandata\Tuning\default.tun
Optimization File: C:\Elandata\Optimize\default.dac
Calibration File: C:\Elandata\System\Dr Saliza (FPSK).cal
Calibration Type: External Calibration

Summary

Intensities

Analyte	Mass	Meas. Intens. Mean	Meas. Intens. RSD	Blank Intensity	Blank Intens. RSD
As	75	615	1.869	76.000	16.079
Pb	208	76111	1.407	1166.742	2.722
Cr	52	49163	2.483	12655.027	2.014
Cd	111	269	0.757	20.889	3.685

Concentration Results

Analyte	Mass	Net Intens. Mean	Conc. Mean	Conc. SD	Conc. RSD	Sample Unit
As	75	539.132	**0.475**	0.01	2.1	ppb
Pb	208	74944.359	**3.658**	0.05	1.4	ppb
Cr	52	36507.825	**3.338**	0.11	3.3	ppb
Cd	111	248.004	**499.141**	4.10	0.8	ppb

Replicates

Repeat 1

Analyte	Concentration
As	0.483671
Pb	3.698829
Cr	3.288844
Cd	502.719229

Repeat 2

Analyte	Concentration
As	0.476627
Pb	3.675860
Cr	3.465977
Cd	494.668457

Repeat 3

Analyte	Concentration
As	0.463713
Pb	3.599018
Cr	3.259795

Quantitative Analysis - Summary Report

Sample ID: 4

Sample Date/Time: Friday, May 03, 2013 09:53:17
Sample Description: Fish 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\Sample\Dr Saliza (FPSK).sam
Method File: C:\Elandata\Method\dr saliza (fpsk).mth
Dataset File: C:\Elandata\DataSet\Dr Saliza (FPSK)\4.012
Tuning File: C:\Elandata\Tuning\default.tun
Optimization File: C:\Elandata\Optimize\default.dac
Calibration File: C:\Elandata\System\Dr Saliza (FPSK).cal
Calibration Type: External Calibration

Summary

Intensities

Analyte	Mass	Meas. Intens. Mean	Meas. Intens. RSD	Blank Intensity	Blank Intens. RSD
As	75	724	2.894	76.000	16.079
Pb	208	59062	1.103	1166.742	2.722
Cr	52	64736	2.435	12655.027	2.014
Cd	111	370	6.413	20.889	3.685

Concentration Results

Analyte	Mass	Net Intens. Mean	Conc. Mean	Conc. SD	Conc. RSD	Sample Unit
As	75	648.029	**0.571**	0.02	3.2	ppb
Pb	208	57895.176	**2.826**	0.03	1.1	ppb
Cr	52	52081.407	**4.762**	0.14	3.0	ppb
Cd	111	348.896	**702.201**	47.73	6.8	ppb

Replicates

Repeat 1

Analyte	Concentration
As	0.586983
Pb	2.856416
Cr	4.600275
Cd	647.633743

Repeat 2

Analyte	Concentration
As	0.550589
Pb	2.792946
Cr	4.809858
Cd	722.775053

Repeat 3

Analyte	Concentration
As	0.574069
Pb	2.827922
Cr	4.876538

Quantitative Analysis - Summary Report

Sample ID: 5

Sample Date/Time: Friday, May 03, 2013 09:54:57
Sample Description: Fish 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\Sample\Dr Saliza (FPSK).sam
Method File: C:\Elandata\Method\dr saliza (fpsk).mth
Dataset File: C:\Elandata\DataSet\Dr Saliza (FPSK)\5.013
Tuning File: C:\Elandata\Tuning\default.tun
Optimization File: C:\Elandata\Optimize\default.dac
Calibration File: C:\Elandata\System\Dr Saliza (FPSK).cal
Calibration Type: External Calibration

Summary

Intensities

Analyte	Mass	Meas. Intens. Mean	Meas. Intens. RSD	Blank Intensity	Blank Intens. RSD
As	75	594	10.143	76.000	16.079
Pb	208	46206	1.744	1166.742	2.722
Cr	52	61006	3.473	12655.027	2.014
Cd	111	646	7.155	20.889	3.685

Concentration Results

Analyte	Mass	Net Intens. Mean	Conc. Mean	Conc. SD	Conc. RSD	Sample Unit
As	75	517.797	**0.456**	0.05	11.6	ppb
Pb	208	45038.851	**2.198**	0.04	1.8	ppb
Cr	52	48350.924	**4.421**	0.19	4.4	ppb
Cd	111	625.356	**1258.613**	93.06	7.4	ppb

Replicates

Repeat 1

Analyte	Concentration
As	0.476627
Pb	2.227810
Cr	4.231575
Cd	1334.651498

Repeat 2

Analyte	Concentration
As	0.395622
Pb	2.153638
Cr	4.618812
Cd	1154.843578

Repeat 3

Analyte	Concentration
As	0.495411
Pb	2.213354
Cr	4.412959

Quantitative Analysis - Summary Report

Sample ID: 6

Sample Date/Time: Friday, May 03, 2013 09:56:36
Sample Description: Fish 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\Sample\Dr Saliza (FPSK).sam
Method File: C:\Elandata\Method\dr saliza (fpsk).mth
Dataset File: C:\Elandata\DataSet\Dr Saliza (FPSK)\6.014
Tuning File: C:\Elandata\Tuning\default.tun
Optimization File: C:\Elandata\Optimize\default.dac
Calibration File: C:\Elandata\System\Dr Saliza (FPSK).cal
Calibration Type: External Calibration

Summary

Intensities

Analyte	Mass	Meas. Intens. Mean	Meas. Intens. RSD	Blank Intensity	Blank Intens. RSD
As	75	636	4.418	76.000	16.079
Pb	208	29600	1.510	1166.742	2.722
Cr	52	83003	1.873	12655.027	2.014
Cd	111	422	3.664	20.889	3.685

Concentration Results

Analyte	Mass	Net Intens. Mean	Conc. Mean	Conc. SD	Conc. RSD	Sample Unit
As	75	560.022	**0.493**	0.02	5.0	ppb
Pb	208	28432.931	**1.388**	0.02	1.6	ppb
Cr	52	70348.039	**6.432**	0.14	2.2	ppb
Cd	111	400.899	**806.862**	31.10	3.9	ppb

Replicates

Repeat 1

Analyte	Concentration
As	0.467235
Pb	1.407501
Cr	6.280508
Cd	773.763976

Repeat 2

Analyte	Concentration
As	0.495411
Pb	1.364344
Cr	6.562127
Cd	811.334851

Repeat 3

Analyte	Concentration
As	0.516543
Pb	1.391439
Cr	6.454833