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CADMIUM CONCENTRATION IN FRESHWATER FISH AND HEALTH RISK ASSESSMENT IN TWO AREAS AT KLUANG, JOHOR

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CADMIUM CONCENTRATION IN FRESHWATER FISH AND HEALTH RISK ASSESSMENT IN TWO AREAS AT KLUANG, JOHOR

BY

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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.

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ABSTRACT

CADMIUM CONCENTRATION IN FRESHWATER FISH AND HEALTH RISK ASSESSMENT IN TWO AREAS AT KLUANG, JOHOR.

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Introduction : Fishes are the main supply of protein besides meat and poultry products and contributed to a large percentage of dietary protein globally. However within this era of industrialization that full of factories that discharge the heavy metal as their side effluents to the river, may caused fishes in the river to contain heavy metal that could give negative effects for health. One of the common heavy metals that can found in fish is cadmium. This research is important to estimate respondents health risks to cadmium detected in freshwater fish since it was frequently consumed by respondents. The health risk assessement is conducted to quantify the probability of the harmful effects of cadmium to the human population. **Objective :** A cross-sectional comparative study was conducted to determine the cadmium concentration in freshwater fish and health risk assessment in two areas which represent polluted and less polluted area. **Instrument & Methodology :** A total of 30 respondents from Kampung Sedohok (near to polluted Sembrong River) and Kampung Punjut (near to less polluted Kahang River) was randomly selected based on the inclusion criteria. A set of pre-tested questionnaire was used to obtain socio-demographic information, fish frequency intake and health status of respondents. Three species of most consumed freshwater fish (N=6) based on the fish frequency questionnaire were caught from selected river by the local fisherman using net method which later were analysed for cadmium concentration by using AAS. The health risk assessment was indicated by Hazard Quotient (HQ) and Lifetime Cancer Risk (LCR) **Results :** All freshwater is detected with Cd from the range of 4.68 to 7.29 mg/kg. The concentration of cadmium is detected higher in polluted river compared to less polluted river that is about ration 2:3. The health risk assessment of respondents is determine and found that there is a significant difference between the health risk that is HQ and LCR of respondents between both Kampung Sedohok and Kampung Punjut **Conclusion :** The fish from polluted Sembrong River showed a slightly higher in cadmium concentration compared to less polluted Kahang River. Otherwise, the health risk (HQ) and (LCR) of respondents were also slightly higher in polluted Sembrong River compared to less polluted Kahang River. Therefore, due to high frequency of fish consumption of relatively high concentration of Cd in fish from Kampung Sedohok, therefore a strong significant relationship was found between fish consumption and risk assessment. **Recommendation :** It is recommended to take along the biological sample in order to make a more confirmation regarding exposure to cadmium.

Key words: Cadmium, freshwater fish, Lifetime Cancer Risk, Hazard Quotient

ABSTRAK

KEPEKATAN KADMIUM DALAM IKAN AIR TAWAR DAN PENILAIAN RISIKO KESIHATAN DALAM DUA KAWASAN DI KLUANG, JOHOR

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Pendahuluan: Ikan adalah merupakan sumber protein utama dan penyumbang kepada peratus pemakanan protein seluruhnya. Walau bagaimanapun, dalam era globalisasi yang penuh dengan kilang-kilang yang telah membuang sisa buangan ke sungai, menjadikan ikan air tawar ini tercemar dengan pelbagai logam berat yang boleh membawa kepada masalah kesihatan manusia. Salah satu logam berat yang terlibat adalah kadmium. Kajian ini penting bagi mengenalpasti risiko kesihatan kepada responden yang makan ikan air tawar ini. **Objektif** : Satu perbandingan kajian keratan rentas telah dijalankan untuk mengenalpasti kepekatan kadmium dalam ikan air tawar dan penilaian risiko kesihatan pendedahan di dalam kawasan yang mewakili kawasan tercemar dan kawasan kurang tercemar. **Alatan dan kaedah** : Sebanyak 30 responden dari Kampung Sedohok (berhampiran sungai tercemar Sembrong) dan Kampung Punjut (berhampiran sungai kurang tercemar Kahang) dipilih secara rawak berdasarkan kriteria terpilih. Satu set soal selidik telah digunakan untuk mendapatkan demografi penduduk, kekerapan pengambilan ikan dan status kesihatan. Tiga spesies ikan air tawar yang paling kerap dimakan oleh responden telah ditangkap dari sungai berkaitan menggunakan kaedah tangkapan jaring. Kepekatan kadmium dianalisis menggunakan AAS. **Keputusan :** Semua ikan air tawar telah dikenalpasti mengandungi kadmium di antara julat 4.68 mg/kg hingga 7.29 mg/kg. Kepekatan kadmium adalah tinggi di sungai tercemar berbanding sungai yang kurang tercemar. Penilaian risiko kesihatan dikenalpasti dan mendapati bahawa terdapat perbezaan yang signifikan diantara risiko kesihatan iaitu HQ dan LCR bagi responden bagi kedua-dua kampung. **Kesimpulan** : Ikan di Sungai Sembrong yang tercemar mengandungi lebih tinggi kadmium berbanding Sungai Kahang yang kurang tercemar. Selain itu, risiko kesihatan HQ dan LCR juga lebih tinggi di di kalangan responden berhampiran sungai tercemar berbanding responden berhampiran sungai yang kurang tercemar.

Kata kunci: Kadmium, ikan air tawar, risiko kanser, darjah bahaya

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TABLE OF CONTENTS

	Pages
DECLARATION	ii
APPROVAL	iii
ACKNOWLEDGEMENT	iv
ABSTRACT	v
ABSTRAK	vi
TABLE OF CONTENTS	vii
LIST OF TABLES	xiv
LIST OF FIGURES	xvi
LIST OF ACRONYMS AND ABBREVIATION	xix

CHAPTER 1 : INTRODUCTION

1.1	Background of Study	1
1.2	Problem Statement	5
1.3	Study Justification	8
1.4	Conceptual Framework	11
1.5	Definition	

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1.5.1 Conceptual Definition 13

Cadmium Concentration in Freshwater Fish

Health Risk Assessment

Adult

1.5.2 Operational Definition 16

Cadmium Concentration in Freshwater Fish

Health Risk Assessment

Adult

1.6 Objectives 19

1.6.1 General Objectives

1.6.2 Specific Objectives

1.7 Hypothesis 20

CHAPTER 2 : LITERATURE REVIEW

2.1 Cadmium 21

2.1.1 Physicochemical properties of Cd

2.2 Sources of Cd Pollution in the environment 24

2.3 Cd concentration in food 25

2.3.1 Foreign studies

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2.3.2 Local studies

2.4 Cd Concentration Outcomes From Oral Exposure 27

2.4.1 Death 28

2.4.2 Immunological and Lymphoreticular Effects 29

2.4.3 Neurological Effects 30

2.4.4 Reproductive Effects 31

2.4.5 Developmental Effects 32

2.4.6 Cancer 32

2.5 Adult 33

2.6 Health Risk Assessment 33

2.6.1 Hazard identification 33

2.6.2 Dose response assessment 34

2.6.3 Exposure assessment 34

2.6.4 Risk characterization 36

CHAPTER 3 : METHODOLOGY

3.1 Study variables 37

3.2 Study location 37

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3.3 Study design 39

3.4 Sampling method 39

3.5 Study population 41

3.5.1 Study sample

3.5.2 Sample size

3.6 Data collection and instrumentation 44

3.6.1 Data collection flow 45

3.6.2 Questionnaire 46

3.6.3 Reagents 49

3.6.4 Apparatus 50

3.6.5 Cd concentration in freshwater fish 52

3.6.6 Health Risk Assessment 68

3.7 Quality control 71

3.7.1 Standard Operating Procedures (SOPs)

3.7.2 Glassware apparatus

3.7.3 Pre test

3.8 Statistical Analysis 73

3.9 Ethical Concern 75

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CHAPTER 4 : RESULTS

4.1	Response rate	76
4.2	Socio-demographic information of respondents	76
4.3	Frequency intake of freshwater fish.	80
4.4	Comparison of frequency intake of freshwater fish	81
4.5	Other possible exposures to Cd	83
4.6	Cd concentration in freshwater fish	91
4.7	Comparison of Cd concentration in freshwater fish in the 2 areas.	95
4.8	Health risk encountered by respondents from cadmium exposure through consumption of freshwater fish.	96
4.9	Comparison of HQ for non-carcinogenic health effects and LCR for carcinogenic health effects.	99
4.10	Relationship between frequency intake of freshwater fish and health risk assessment in the two areas.	101

CHAPTER 5 : DISCUSSIONS, CONCLUSION, RECOMMENDATION

5.1	Discussion	103
5.1.1	Socio-demography of the respondents.	105
5.1.2	Comparison of frequency intake of freshwater fish	106
5.1.3	Other possible sources of cadmium	107

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5.1.4 Cadmium concentration of freshwater fish. 108

5.1.5 Comparison of Cd concentration in freshwater fish in the 2 areas. 111

5.1.6 Health risk encountered by respondents from cadmium exposure through consumption of freshwater fish. 113

5.1.7 Comparison of HQ for non-carcinogenic health effects and LCR for carcinogenic health effects 113

5.1.8 Relationship between frequency intake of freshwater fish and health risk assessment in the two areas. 115

5.1.9 Study strength 117

5.1.10 Study limitation 118

5.2 Conclusion 119

5.3 Recommendation 120

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REFERENCES 122

APPENDIX 1 : Ethical Committee Consent Letter

APPENDIX 2 : Approval letter from JAKOA Ampang

APPENDIX 3 : Information sheet

APPENDIX 4 : Respondent sheet

APPENDIX 5 : Questionnaires

APPENDIX 6 : Result of AAS

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LIST OF TABLES

			Pages
Table 3.1	:	The data of freshwater fish from Sungai Sembrong.	55
Table 3.2	:	The data of freshwater fish from Sungai Kahang.	55
Table 3.3	:	The weight for each sample (before digestion)	60
Table 3.4	:	The mean volume of each sample after digestion	61
Table 4.1a	:	Socio-demographic information of the respondents.	78
Table 4.1b	:	Socio-demographic information of the respondents.	79
Table 4.2	:	The comparison of frequency intake of freshwater fish among respondents.	82
Table 4.3	:	The source of freshwater fish for both Kampung Sedohok and Kampung Punjut	83
Table 4.4	:	Other possible sources of cadmium	84
Table 4.5	:	Prevalence and mean frequency of the top ten daily consumed food among respondents from Kampung Sedohok.	86
Table 4.6	:	Prevalence and mean frequency of the top ten daily consumed food among respondents from Kampung Punjut.	87
Table 4.7	:	Prevalence and mean frequency of the top ten weekly consumed food among respondents from Kampung Sedohok.	88
Table 4.8	:	Prevalence and mean frequency of the top ten food items consumed weekly by the adult populations for Kampung Punjut	89

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Table 4.9	:	Parameters used to convert raw data of AAS to actual reading of Cd concentration.	92
Table 4.10	:	The data for mean concentration of Cd in freshwater fish samples.	94
Table 4.11	:	Comparison of the Cd concentration in the freshwater fish of Kampung Sedohok and Kampung Punjut.	95
Table 4.12	:	Mean and standard deviation of the value ADD, LADD, HQ, and LCR.	98
Table 4.13	:	Comparison of hazard quotient (HQ) and Lifetime cancer risk (LCR) of the respondents between the two areas.	100
Table 4.14	:	Spearman's rho test between freshwater fish frequency intake and health risk LCR and HQ.	102

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LIST OF FIGURES

			Pages
Figure 1.1	:	Sembrong River in Kluang, Johor.	4
Figure 1.2	:	Kahang River in Kluang, Johor.	4
Figure 1.3	:	The tube effluents from one of the factory nearby that discharged into the Sembrong River.	7
Figure 1.4	:	Conceptual framework of cadmium contamination from dietary intake to human.	12
Figure 1.5	:	The Atomic Absorption Spectroscopy	17
Figure 2.1	:	A small ball of cadmium metal, with a coin for size reference	22
Figure 3.1	:	Map of Kluang, Johor.	38
Figure 3.2	:	A location at Sembrong River where the fisherman caught the freshwater fish.	39
Figure 3.3	:	Location of Kahang River that near to Kampung Punjut.	40
Figure 3.4	:	Location of Sembrong River that near to Kampung Sedohok.	41
Figure 3.5	:	The formula of sample size that was used in the study.	43
Figure 3.6	:	Data collection flow of Cd concentration in freshwater fish and health risk assessment among adults respondents in two areas at Kluang, Johor.	45
Figure 3.7	:	The interview session with respondents of Kampung Sedohok	47
Figure 3.8	:	Seca weight to measure the weight of repondents	51

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Figure 3.9	:	Seca height to measure the height of respondents.	51
Figure 3.10	:	The cast net that was used to catch fishes.	54
Figure 3.11	:	The example of *Lampan Jawa* species.	56
Figure 3.12	:	The example of *Terubol* species	56
Figure 3.13	:	The example of *Jelawat* species.	57
Figure 3.14	:	The powdered sample of freshwater fish was grinded by using mortar and pestle.	59
Figure 3.15	:	The muscles of freshwater fish was being cut	61
Figure 3.16	:	The standard solution of cadmium.	62
Figure 3.17	:	The mother solution of cadmium.	65
Figure 3.18	:	Samples for analysis of freshwater fishes.	66
Figure 3.19	:	The tube that contain samples.	67
Figure 3.20	:	The pre-test questionnaire that was done at nearby village that is Kampung Tanah Runtuh	73
Figure 4.1	:	The frequency intake of freshwater fish among respondents	81
Figure 4.2	:	Cd concentration in freshwater fish of both Kampung Sedohok and Kampung Punjut.	93
Figure 4.3	:	The pie-chart of the mean cadmium concentration.	95
Figure 5.1	:	The access to the Kampung Sedohok.	104
Figure 5.2	:	The wood manufacturing near to Sembrong River.	109

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Figure 5.3	:	The unknown substances found at Sembrong River	109
Figure 5.4	:	The oil palm manufacturing nearby to Sembrong River and Kampung Sedohok.	110
Figure 5.5	:	The compost fertilizer in the area of Kampung Sedohok and Sembrong River.	110
Figure 5.6	:	The abrasive substances found near to Sembrong River.	112

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LIST OF ACRONYMS AND ABBREVIATIONS

Cd	Cadmium
HIA	Health Impact Assessment
EIA	Environment Impact Assessment
USEPA	United States Environmental Protection Agency
AAS	Atomic Absorption Spectroscopy
SPSS	Statistical Packages for Social Sciences
SOP	Standard Operating Procedure
IFS	International Fisheries Symposium
HQ	Hazard Quotient
LCR	Lifetime Cancer Risk
INS	International/ National Standard

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CHAPTER 1

INTRODUCTION

1.1 Background of the study

Foods are the important elements in living things especially to humans. We eat foods in order to survive and grow. There are many types and form of foods that are available nowadays, starting from the raw foods, ready to eat foods, fast foods, preservative foods and many other type of foods. We can find foods everywhere in the markets, supermarkets, stalls or even side of the road.

Fishes are the main supply of protein besides meat and poultry products and contributed to a large percentage of dietary protein globally. In most Asian countries, especially in Southeast Asia like Thailand, Indonesia and Malaysia fish is taken as the main dish of their diet. It is particularly valuable for providing food with nutritional value in the form of protein, and minerals such as calcium, phosphorus, iron and copper (Alina *et. al*., 2012).

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Besides protein, fish is also high in essential fatty acids (EFAs), known aseicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) that are important to the diet. In addition, consumption of fatty fish is recommended by nutritionist as a mean to prevent cardiovascular diseases (Siscovick *et. al.*, 2012)

However within this era of industrialization that full of factories that discharge the heavy metal as their side effluents to the river, may caused fishes in the river to contain heavy metal that could give negative effects for health (Rosnita, 2008). It is mentioned that diet and food of animals origin are the most predominant sources (>90%) of heavy metals and other chemical contaminants to human. In many countries, industrial wastes, geochemical structure and mining of metals create a potential source of heavy metals pollution in the aquatic environment due to their toxicity and accumulation behavior (Sivaperumal *et. al.*, 2007).

Although, fish and shellfish only represent small percentage (10%) of these contaminants, these foods are one of the major routes of the contaminants in the body. Some of the metals are essential to the human body but some of them can cause harm to the body (Alina *et. al.,* 2012).

The sample from this study was collected from self-fishing at the two areas which located to Sembrong River and Kahang River. The both river were chosen based on the category of polluted (Sembrong River) and less polluted (Kahang

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River). Otherwise, there are a lot of manufacturing plants that are build along the Sembrong River compared to Kahang River. Most of these development of the manufacturing industrialization used cadmium in their production.

Therefore, this study would determine the cadmium concentration in the freshwater fish in two areas at Kluang Johor that are at Sembrong River (Figure 1.1) and Kahang River (Figure 1.2) and as well as the calculation of health risk assessment of the respondents from the related villages that consume the freshwater fish as their main diet (Azman, 2007)

One of the common heavy metals that can be found in fish is cadmium. As a fact, cadmium is used in galvanising and electroplating in batteries, in electrical conductors, in the manufacture of alloys, pigments and plastics, and in the stabilisation of phosphate fertilisers (Pubmed, 2009).

It is also a prevalent environmental contaminant due to its release from metal smelters. Otherwise, exposure to cadmium and other such "metalloestrogens" could result in an increased rate of breast and prostate cancer. In general, exposure to cadmium occurs primarily through food, cigarette smoking and sometimes drinking water (Utusan Malaysia, 2009).

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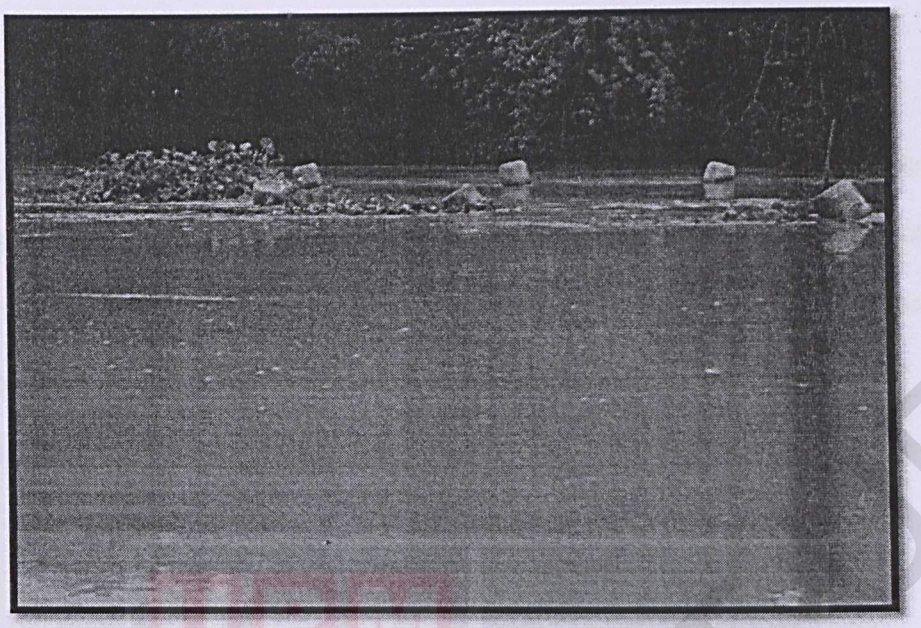


Figure 1.1 : Sembrong River in Kluang, Johor.



Figure 1.2 : Kahang River in Kluang, Johor.

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1.2 Problem statement

Fisheries is one of the most important food production sectors in supplying proteins to the human population. According to Food and Agriculture Organisation (FAO), world fisheries production in 2008 touched 146 million tonnes and a record value of 103 billion USD. Due to the increasing health consciousness of the consumers, seafood demands increase drastically during the past decade (Wordpress, 2011).

Therefore, in order to considering this issues, present research was initiated to determine the bioaccumulation level of hazardous metals in some commercially important fishes from South West coast of Peninsular Malaysia, covering 3 states of Johor, Melaka and Negeri Sembilan (Kamaruzzaman *et. al*., 2011).

There is also a research finding about the heavy metal in Johor river that is at the Skudai River, Johor Bahru. Some research was conducted in September 2005, discovered that trend of heavy metals in water of the Skudai River system was Zn>Cu>Ni>Cr>As>Pb>Hg>Cd (Nurhashimah, 2007).

Through pollution of Skudai River, it is heard also about the news regarding the heavy metal pollution in a river at the Mengkibol River, Kluang. The Mengkibol's River pollution is actually occurring from the discharging effluents of the industrialization of textile from the Pasir Gudang, Johor Bahru. The effluent from

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the textiles factory at the Pasir Gudang had made the Mengkibol River to be affected by the heavy metal's discharged from it (Nurhashimah, 2007).

The Mengkibol River is one of the branch of Sembrong River, Kluang. Therefore, the contamination from Mengkibol River may flow to the Sembrong River. In fact, Sembrong River is the most longest river in Kluang, Johor and this study concerned about this river because, there are *Orang Asli* nearby that live and consumed the freshwater fish from this river. The culture of *Orang Asli* usually will choose to eat freshwater fish as their main food source instead of marine fish.

However, there are a lot of manufacturing plants that are build along the Sembrong River compared to Kahang River such as, oil palm factory, rubber plantation, gas of methane project, compost fertilizer, treatment plant, Kimberly Clark, and Huggies. Most of these manufacturing plants used cadmium in their production. The effluents from the production was discharged into the river as shown in Figure 1.3. So, that is why the Sembrong River was be sured about its contamination compared to Kahang River.

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Figure 1.3 : The tube effluents from one of the factory nearby that discharged into the Sembrong River.

Clearly, of the anthropogenic sources of cadmium, phosphate fertilisers, fossil fuel combustion, and some industrial activities contribute far more to human cadmium exposure than production, use and disposal of cadmium products and incineration of all cadmium-containing materials. However, as shown earlier, this observation should be put in the perspective of average daily cadmium intakes well below those necessary to ensure human health. Thus no action seems required to reduce cadmium from its existing levels (International Cadmium Conference, 2011).

Therefore, this study would determine the cadmium concentration in the freshwater fish at Sembrong River that can be classified as polluted river and also Kahang River that can be classified as less polluted river. The exceeded permitted

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level of the cadmium in the fish, may directly accumulated into the food chain and then contaminated the body and thus may give harmful effects to the health of human. This study also would accessed the health risk of community who consumed freshwater fish from the two areas.

1.3 Study justification

A main route for the exposure to the cadmium is through the ingestion or consumption of food especially by humans. Humans eat the foods that are from aquatic life or even terrestrial foods. Cadmium is therefore naturally present in air, water, soil and foodstuffs (Webelement, 2012).

In this study, cadmium is chosen as the parameter in terms of determining its concentration in the freshwater fish that could give harmful effects to the health of respondents. This is because, cadmium is an extremely toxic metal commonly found in industrial workplaces. Due to its low permissible exposure limit, overexposures may occur even in situations where trace quantities of cadmium are found.

The aim of this study is to determine the cadmium concentration in freshwater fish and health risk assessment among adults in two areas at Kluang, Johor.

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The study is done because, most of the study conducted on the determination of heavy metal in the food are less concern about the health risk to the human body. The health risk assessment is conducted to quantify the probability of the harmful effects of cadmium to the human population.

The freshwater is chosen instead of marine water because, there are lack of previous study regarding freshwater fish. The study regarding the marine fish, is so much available and the availability for the freshwater fish nowadays is also quite limited as because of the development of the industry or other man-made activity that have been destroy the natural resources of the freshwater. So, this study would discover the healthiness of the fish from the cadmium, in this limited freshwater (Freshwaterlife, 2012).

Industrilization mostly occurs at the west coast of Peninsular Malaysia. One of it is Johor. Kluang is a landlock and no seafront at Johor. Regarding the Department of Fisheries Kluang Johor, Kluang is the center and famous for the freshwater fish in the state of Johor. Otherwise, according to Department of Environment, (2012) some of the rivers at Kluang, Johor are already listed as the polluted and less polluted river according to their class. The Sembrong River is in class III while Kahang River is in class II. Moreover, the Department of Fisheries Kluang Johor itself suggested to do the study of fish from this both river. It is really suits for our study in order to compare the cadmium concentration in the freshwater fish in the polluted and less polluted river that is at Sembrong River and Kahang

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River respectively and then accessing health risk of respondents. Furthermore, this both river were separated river and meet at the same river that is at Madek River.

This study also choose to sample the flesh of fish as this part is usually consumed by the human compared to other part of fish. Otherwise, most studies have been concentrate only on the accumulation of heavy metal in the edible part (muscle), in view of the fact that it is the main fish part that is consumed by human beings (Keskin *et. al.*, 2007).

Therefore, this study is important because in order to make sure that whether the level of cadmium is exceed the limit of the standard given by the government or not. Then, data and findings of this study are consistently important to indicate the safety of local freshwater fish products and could also be used to economically protect the consumers that buy the freshwater fish in the area as well as protected the health of *Orang Asli*. Next, this can be used in order to trigger the effects of the high level of cadmium to the health of humans.

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1.4 Conceptual framework

Figure 1.4 shows the conceptual framework which the highlighted area indicated the study scope that is involved in this study. The figure shows the flow of how the cadmium may found in the food chain of the human body and thus give harmful effects to the human health.

The cadmium can enter our body by the dietary intake. The dietary intake may be sourced from the animal sources or non-animal sources. Then, the animal sources that are available in river is highlighted as this study will discuss on the freshwater fish. For nonsmokers, food is generally the largest source of cadmium exposure. Cadmium levels in some foods can be increased by the application of phosphate fertilizers or sewage sludge to farm fields (USEPA, 2007)

The contaminated freshwater fish is then eaten by human through ingestion. Therefore, this study will see how the cadmium may caused a result of health effects that can disrupt the normal functions of the human body. Cadmium also has the carcinogenic and non-carcinogenic health effects.

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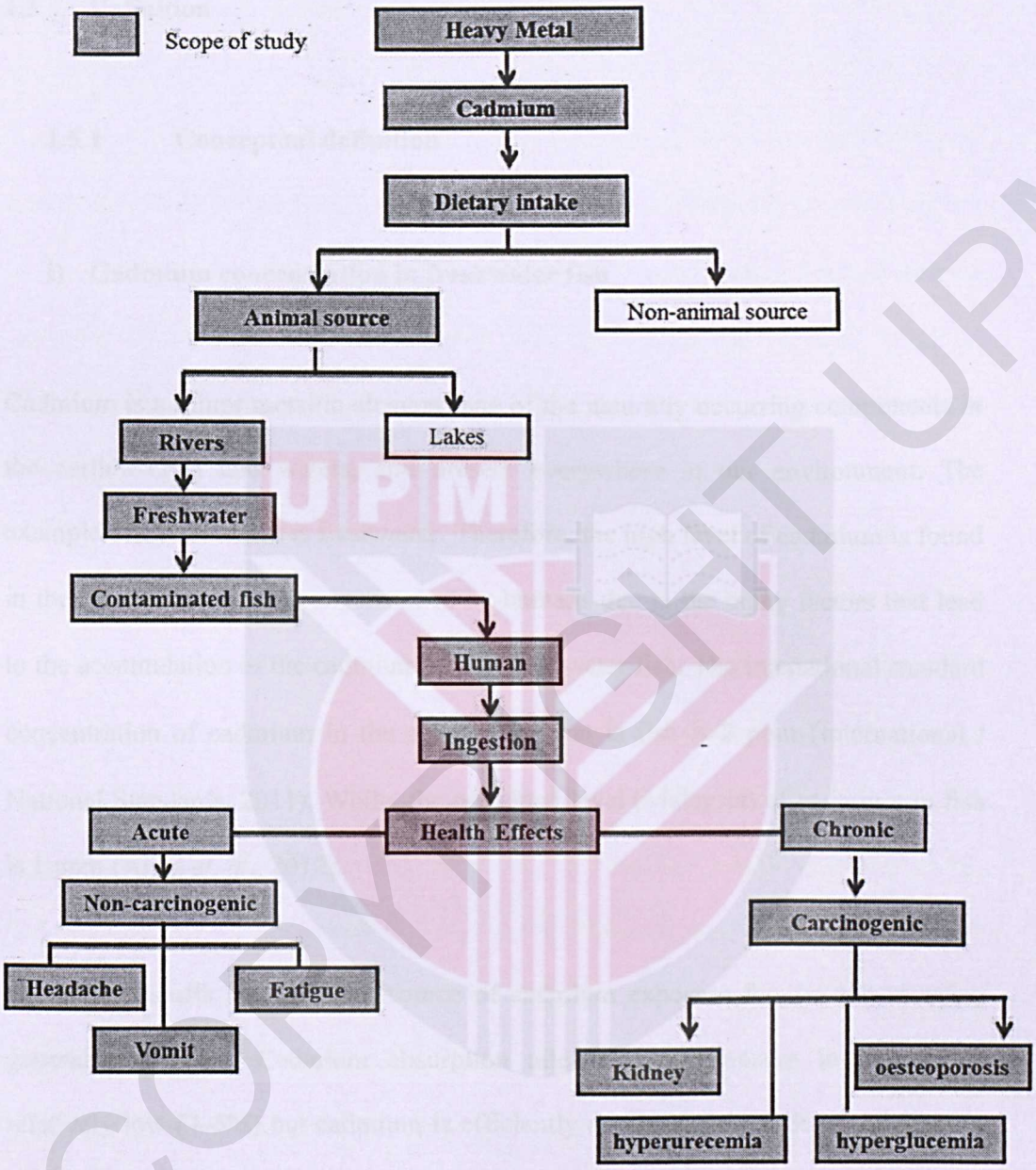


Figure 1.4 : Conceptual framework of cadmium contamination from dietary intake to human.

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1.5 Definition

1.5.1 Conceptual definition

i) Cadmium concentration in freshwater fish

Cadmium is a minor metallic element, one of the naturally occurring components in the earth's crust and waters, and present everywhere in our environment. The example of environment is freshwater. Therefore, the high level of cadmium is found in the freshwater fish that is consumed by humans due to the many factors that lead to the accumulation of the cadmium in this freshwater fish. The intenational standard concentration of cadmium in the aquatic life that is fish is 2 ppm (International / National Standards, 2011). While, the permitted level (Malaysia) of cadmium in fish is 1 ppm (Alina *et. al*., 2012).

Foodstuffs are the main source of cadmium exposure for the non-smoking general population. Cadmium absorption after dietary exposure in humans is relatively low (3–5%) but cadmium is efficiently retained in the kidney and liver in the human body, with a very long biological half-life ranging from 10 to 30 years (EFSA, 2012).

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ii) Health Risk Assessment

Health Risk Assessment (HRA) 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. The exposure to the high concentration of cadmium in the intake and consumption may lead to various types of illnesses. Human health risk assessment includes 4 basic steps, that are, hazard identification, dose-response assessment, exposure assessment and risk characterization (USEPA, 2012).

Hazard Identification is the process of determining whether exposure to a stressor can cause an increase in the incidence of specific adverse health effects (e.g., cancer, birth defects) and whether the adverse health effect is likely to occur in humans. In the case of chemical stressors, the process examines the available scientific data for a given chemical (or group of chemicals) and develops a weight of evidence to characterize the link between the negative effects and the chemical agent.

A dose-response relationship describes how the likelihood and severity of adverse health effects (the responses) are related to the amount and condition of exposure to an agent (the dose provided). Although this webpage refers to the "dose-response" relationship, the same principles generally apply for studies where the exposure is to a concentration of the agent (e.g., airborne concentrations applied in

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inhalation exposure studies), and the resulting information is referred to as the "concentration-response" relationship. The term "exposure-response" relationship may be used to describe either a dose-response or a concentration-response, or other specific exposure conditions.

Exposure assessment considers both the exposure pathway (the course an agent takes from its source to the person(s) being contacted) as well as the exposure route (means of entry of the agent into the body). The exposure route is generally further described as intake (taken in through a body opening, e.g. as eating, drinking, or inhaling) or uptake (absorption through tissues, e.g. through the skin or eye).

A risk characterization conveys the risk assessor's judgment as to the nature and presence or absence of risks, along with information about how the risk was assessed, where assumptions and uncertainties still exist, and where policy choices will need to be made. Risk characterization takes place in both human health risk assessments and ecological risk assessments.

iii) Adult

Adult is a human being or living organism that is of relatively mature age, typically associated with sexual maturity and the attainment of reproductive age. In human context, the term has other subordinate meanings associated with social and legal concepts; for example, a legal adult is a legal concept for a person who has attained

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the age of majority and is therefore regarded as independent, self-sufficient, and responsible (contrast with "minor").

Adulthood can be defined in terms of physiology, psychological adult development, law, personal character, or social status. Malaysian Adult Age Act 1971 has defined adult age in Malaysia is 18 years and above. Norimah *et. al*. (2008) define population adult in Malaysia as 18 to 59 years old.

In this study, the adults respondents are both male and female, and they also do not have any chronic diseases.

1.5.2 Operational definition

i) Cadmium concentration in freshwater fish

The sample of freshwater fish were caught by the fisherman at the Sembrong River and Kahang River respectively. The species of freshwater fish was taken based on the frequency intake by the respondents. The species of the freshwater for both river are the same. There were 3 species of freshwater fish taken from both river that were *Lampam* species (*Puntius gonionotus*), *Jelawat* species (*Leptobarbos hoevenii*) and *Terubol* species (*Osteochilus hasseltii*). For each species of freshwater fish taken, there were 3 pieces caught. Then, each of the pieces, it was triplicated.

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Then, the sample of the freshwater fishes were analysed. Extraction of freshwater fishes' sample drawn by using the wet acid digestion. Then, the cadmium concentration was determined using Atomic Absorption Spectroscopy (AAS), determination of cadmium The cadmium concentration of the freshwater fish was then averaged. The value of the average cadmium concentration is written as mg/L.

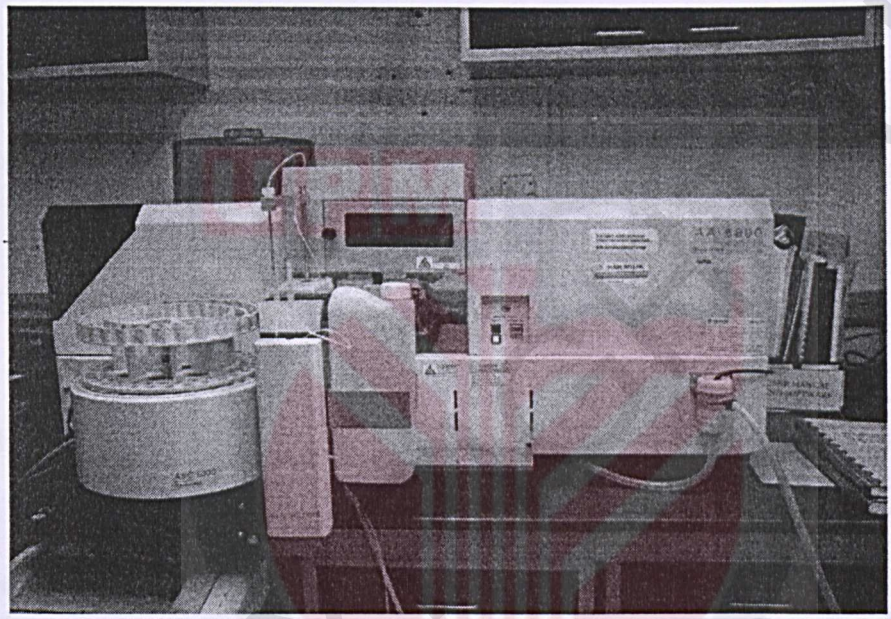


Figure 1.5 : The Atomic Absorption Spectroscopy

The Cadmium concentration in the extract need to be calculated by using formula in order to obtain the final cadmium concentration.

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ii) Health Risk Assessment

The Health Risk Assessment (HRA) was evaluated from analysing of the questionnaires regarding the daily intake of the freshwater fish. Therefore, the calculation of the lifetime average daily dose (LADD), average daily dose (ADD), lifetime cancer risk (LCR) and hazard quotient (HQ) are calculated.

The LCR represent carcinogenic health effects and HQ represent non-carcinogenic health effects.

iii) Adult

This study only take the adult respondents at the Kampung Sedohok and Kampung Punjut from the range of age of 18 to 59 years old. It was obtain from the survey that had been done at the related village.

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1.6 Objectives

1.6.1 General objective

To determine the cadmium concentration in freshwater fish and health risk assessment among adults in two areas at Kluang, Johor.

1.6.2 Specific objectives

- To determine the socio-demography of the respondents.
- To compare the frequency intake of freshwater fish in two areas.
- To compare the cadmium concentration of freshwater fish between two areas.
- To compare health risk assessment of the respondents between two areas.
- To determine the relationship between the frequency intake and health risk assessment in two areas.

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1.7 Hypothesis

- There is a significant difference of the frequency intake of freshwater fish between two areas.
- There is a significant difference of the cadmium concentration of freshwater fish between two areas.
- There is a significant difference of the health risk assessment of the respondents between the two areas.
- There is a significant relationship between the frequency intake and health risk assessment between two areas.

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CHAPTER 2

LITERATURE REVIEW

2.1 Cadmium

Cadmium is a metal found in the earth's crust, associated with zinc, lead, and copper ores. Pure cadmium is a soft, silver-white metal. Cadmium chloride and cadmium sulfate are soluble in water. In the United States, for nonsmokers the primary source of cadmium exposure is from the food supply.

A small amount of the cadmium in food and water (about 1–10%) will enter your body through the digestive tract. Figure 2.1 below shows a small ball of cadmium metal, with a coin for size reference. If people do not have enough iron or other nutrients in their diet, they are likely to take up more cadmium from their food than usual. Most of the cadmium that enters body goes to kidney and liver and can remain there for many years. A small portion of the cadmium that enters body leaves slowly in urine and feces (ATSDR, 2008).

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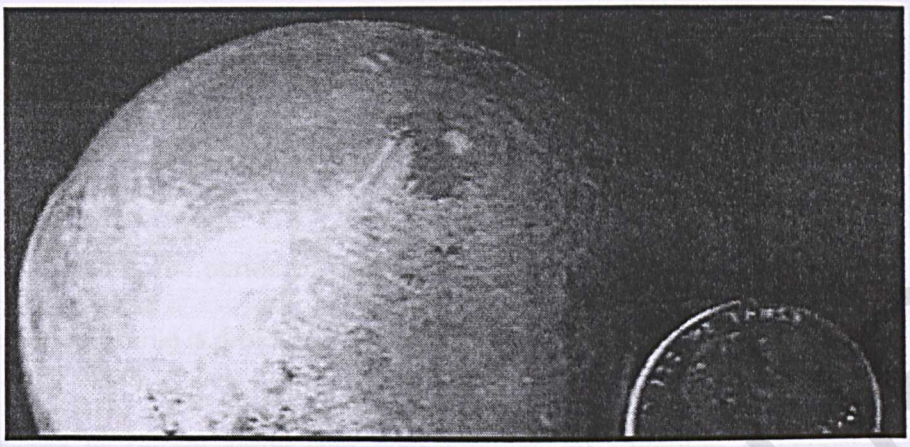


Figure 2.1 : A small ball of cadmium metal, with a coin for size reference. (Source : Chemicool, 2012)

2.1.1 Physicochemical properties of Cd

Cadmium's chemistry is similar to zinc's and they are found in the same ores. Zinc sits above cadmium in the periodic table. Like mercury below it, cadmium and its compounds are highly toxic.

Some chemists don't regard cadmium as a true transition metal, because it has a full shell of d electrons. The IUPAC definition of a transition metal is one whose atoms have an incomplete d sub-shell, or which can give rise to cations with an incomplete d sub-shell. By this definition, cadmium, whose ions have incomplete d electron shells, is a transition metal (Webelement, 2012).

Cadmium and its compounds are highly toxic. Cadmium is a soft, malleable, ductile, bluish-white metal, which is easily cut with a knife. It is an excellent electrical conductor and shows good resistance to corrosion and attack by chemicals. It is similar in many respects to zinc in its chemical properties. Cadmium tarnishes in

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air and is soluble in acids but not in alkalis.The metal burns in air to form brown cadmium oxide (CdO).

Cadmium (Cd) has the atomic number of 48, and its group in the periodic table is 12. Otherwise, the period of cadmium in the periodic table is 5, while the block in the periodic table is d-block (Webelement, 2012).

Exposure to certain forms and concentrations of cadmium is known to produce toxic effects on humans. Long-term occupational exposure to cadmium at excess concentrations can cause adverse health effects on the kidneys and lungs. Adverse human health effects have generally not been encountered under normal exposure conditions for the general population except in areas of historically high cadmium contamination (Badsha, 2006).

The potential risks from cadmium exposure have been extensively studied, and are now tightly controlled by occupational exposure standards, regulations for cadmium in ambient air, water and soil, and legislation covering cadmium emissions, labeling and disposal of cadmium-containing products, and impurity levels in other products such as fossil fuels, fertilizers and cement (International Cadmium Conference, 2011).

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2.2 Sources of Cd Pollution in environment

According to ATSDR (2008), cadmium has been widely dispersed into the environment through the air by its mining and smelting as well as by other man-made routes that are through usage of phosphate fertilizers, presence in sewage sludge, and various industrial uses such as NiCd batteries, plating, pigments and plastics.

The most important sources of airborne cadmium are smelters. Other sources of airborne cadmium include burning fossil fuels such as coal or oil and incineration of municipal waste such as plastics and nickel-cadmium batteries. Cadmium may also escape into the air from iron and steel production facilities. Cadmium is used mainly in metal plating, producing pigments, NiCd batteries, as stabilizers in plastics, and as a neutron absorbent in nuclear reactors (Caslab, 2012).

Cadmium concentrations in drinking water supplies are typically less than 1 microgram per liter (µg/L) or 1 part per billion (ppb). Groundwater seldom contains high levels of cadmium unless it is contaminated by mining or industrial wastewater, or seepage from hazardous waste sites. Soft or acidic water tends to dissolve cadmium and lead from water lines; cadmium levels are increased in water stagnating in household pipes. These sources have not been reported to cause clinical cadmium poisoning, but even low levels of contamination add to the body's accumulation of cadmium (Caslab, 2012).

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From the soil, certain plants (tobacco, rice, other cereal grains, potatoes, and other vegetables) take up cadmium more rapidly than they do other heavy metals such as lead and mercury (Caslab, 2012).

Cadmium is also found in meat, especially sweetmeats such as liver and kidney. In certain areas, cadmium concentrations are elevated in shellfish and mushrooms (Factsheets, 2012).

2.3 Cd concentration in food

2.3.1 Foreign studies

A research was done to determine the cadmium concentrations in ordinarily consumed foods and beverages in Japan. The cadmium concentration is determined by using Atomic Absorption Spectroscopy (AAS). Cd in ten typical Japanese menus for the study were estimated to range from 11.9 to 184.14 µg/d (Yuriko, 2002)

A study done at the Matlab, showed that both rice and vegetables contained cadmium. The presence of cadmium in rice and vegetables in Matlab could be due to the use of chemical fertilizers, e.g. triple superphosphate, and pesticides (composed of cadmium or its derivatives) for the cultivation of paddy and vegetables. Toxic metal deposition in urban and rural areas is a problem because of industrial wastage,

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sewage sludge, chemical fertilizers, and pesticides. As a consequence, grazing animals are exposed to pollutants deposited on pastures. Thus, humans are additionally exposed to cadmium-contaminated animal products, especially from meat and milk (Caslab, 2012).

According to EFSA (2012), to assess cadmium dietary exposure, the occurrence data and the consumption data as reported in the EFSA's Concise European Food Consumption Database were used. National food consumption dietary surveys were used to estimate the consumption pattern of specific sub-groups such as vegetarians and children. The food groups that contributed to the major part of the dietary cadmium exposure, primarily because of the high consumption, were cereals and cereal products, vegetables, nuts and pulses, starchy roots or potatoes, and meat and meat products.

Due to their high consumption of cereals, nuts, oilseeds and pulses, vegetarians have a higher dietary exposure of up to 5.4 µg/kg b.w. per week. Regular consumers of bivalve molluscs and wild mushrooms were also found to have higher dietary exposures of 4.6 and 4.3 µg/kg b.w. per week, respectively. Tobacco smoking can contribute to a similar internal exposure as that from the diet. House dust can be an important source of exposure for children (EFSA, 2012).

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2.3.2 Local studies

A study was done along the Straits of Malacca found that Cd concentration detected in the marine and shellfish. Cd was highest in cockles at both sampling time in the amount of 0.470 ± 0.00 µg/ g and 0.251 ± 0.00 µg/ g of wet weight of samples, respectively. This could be due to the fact that the habitat of Anadara granosa (cockles) that are living at the bottom of sea, nearly to the sediment where various kinds of hazardous and toxic substances are accumulated (Kamaruzzaman *et al.*, 2011).

There is a research found that, bivalve mollusc species had a high capacity and propensity to concentrate pollutants. Furthermore, the environmental conditions and seasonal fluctuations of lipids may influence the heavy metals to accumulate (Alina *et. al.*, 2012).

2.4 Cd concentration outcomes from oral exposure

Information on health effects of oral exposure to cadmium in humans is derived mainly from studies of residents living in cadmium-polluted areas. Cadmium exposure in these populations is often estimated by blood or urinary cadmium levels. Exposure in these cases occurs primarily through the diet, but smokers in these cohorts are also exposed to cadmium by inhalation. When evaluating oral exposure studies, smoking was treated as a confounding variable rather than an exposure route

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because of the large number of toxic compounds (in addition to cadmium) present in cigarette smoke, and because the primary concern is effects attributable to cadmium (Handbook of Cadmium, 2012)

Cadmium is more readily found in the free ionic form in water, while in food, the cadmium ion generally exists in a complex with a variety of ligands, including proteins such as metallothionein. Experimental studies in animals have generally used soluble salts of cadmium (such as cadmium chloride) for food, drinking water, and gavage exposures. The toxicological properties of the cadmium ion do not appear to depend on the counter ion, although absorption may be significantly affected by protein complexes (ATSDR, 2012).

2.4.1 Death

Intentional ingestion of cadmium has been used as a means of suicide, causing death due to massive fluid loss, edema, and widespread organ destruction. The doses ingested in two known fatal cases were estimated to be 25 mg Cd/kg from cadmium iodide and 1,840 mg Cd/kg from cadmium chloride. Time to death after cadmium iodide ingestion was 7 days and 33 hours after ingestion of the cadmium chloride (ATSDR, 2007).

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2.4.2 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans after oral exposure to cadmium. Numerous studies in rats, mice, and monkeys have established the capability of cadmium to affect the immune system, but the clinical significance of the effects is not clear. In mice, intermediate-duration oral exposure to cadmium has been shown to increase resistance to viral infection, to be without effect on natural or acquired resistance to infection, and to increase mortality from virally-induced leukemia.

Oral cadmium exposure has also been found to suppress the humoral immune response of mouse splenic cells to sheep red blood cell antigen in 6-week-old mice, but not in 12-month-old mice. The author suggests that "natural" age-related immune system dysfunction masked any cadmium suppressive effect in the 12-month-old mice, and that immunotoxicological investigations in aged models appear to be a poor indicator of immune response in the general population. Oral cadmium exposure has also been found to increase the cell-mediated immune response of monkeys, to induce anti-nuclear antibodies in mice, to increase circulating leukocytes in female rats, and to exhibit time-dependent inhibitory and stimulative effects or no effect on natural killer cell activity in rats (ATSDR, 2008).

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2.4.3 Neurological Effects

A study have reported an association between environmental cadmium exposure and neuropsycho-logical functioning. These studies used hair cadmium as an index of exposure. End points that were affected included verbal IQ in rural Maryland children, acting-out and distractibility in rural Wyoming children, and disruptive behavior in Navy recruits. The usefulness of the data from these studies is limited because of the potential confounding effects of lead exposure; lack of control for other possible confounders including home environment, caregiving, and parental IQ levels; and an inadequate quantification of cadmium exposure (Timothy *et. al.*,2013)

Although cadmium-induced neurotoxicity has not been clearly demonstrated in human studies, it has been observed in animal studies. Both a single oral exposure and intermediate-duration exposure of adult rats to cadmium resulted in significantly decreased motor activity. Intermediate-duration oral exposure to cadmium has also been reported to cause weakness and muscle atrophy, induce aggressive behavior, induce anxiety as manifested by increased passive avoidance behavior and by increased ethanol consumption, and alter brain biogenic amine content and enzyme activities (ATDSR, 2007).

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2.4.4 Reproductive Effects

Several studies have examined the possible association between increased cadmium exposure and male reproductive toxicity; however, most studies focused on sex steroid hormone levels and the results appear to be inconsistent. ATSDR (2007) found significant associations between increasing blood cadmium levels and increasing levels of serum luteinizing hormone, follicle stimulating hormone, prolactin, and testosterone among infertile men (sperm counts <20 million/cm3 or no spermatozoa in semen).

A significant association between increased blood cadmium levels and increased serum testosterone was also found in a group of workers with slight to moderate lead exposure; however, neither study controlled for smoking. A study by Alica *et al*. (2009) found significant associations between blood cadmium levels and increased serum estradiol, follicle stimulating hormone, and testosterone levels in infertile men after adjusting for age, smoking, alcohol consumption, and biomarkers of lead, copper, zinc, and selenium.

In contrast, a study of Chinese men living in areas with high levels of cadmium in rice did not find significant correlations between urinary or blood cadmium levels and serum testosterone, follicle stimulating hormone, or luteinizing hormone levels after adjusting for BMI, age, smoking, and alcohol consumption. However, they did find that the prevalence of abnormally elevated serum

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testosterone levels (>95th percentile for controls) increased with exposure to cadmium (ATSDR, 2008).

2.4.5 Developmental Effects

The most sensitive indicator of developmental toxicity of cadmium in animals appears to be neuro-behavioral development. Offspring of female rats orally exposed to cadmium at a dose of 0.04 mg/kg/day prior to and during gestation had reduced exploratory locomotor activity and rotorod performance at age 2 months (Baranski et al. 1983). Pups from dams exposed to 0.7 mg/kg/day during gestation had significant delays in cliff aversion and swimming behavior. Locomotor activity was significantly increased.

In post-weaning measurements, locomotor activity was significantly decreased in treated groups at 60 days of age; conditioned avoidance behavior was also significantly decreased when tested at 60 and 90 days of age (ATSDR, 2007).

2.4.6 Cancer

Epidemiology studies have examined the possible association between exposure to elevated levels of cadmium and overall cancer rates or specific cancer rates. Older studies have examined cancer rates among residents of cadmium polluted areas but did not estimate cadmium exposure levels. No significant increase in cancer rates

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was found among residents of a cadmium-polluted village in England (Juhua *et al.* 2011) or in prostate, kidney, or urinary tract cancer among residents of a cadmium-polluted area of Belgium (ATSDR, 2007).

2.5 Adult

Malaysian Adult Age Act 1971 has defined adult age in Malaysia is 18 years and above. Norimah et. al. (2008) define adult population in Malaysia is 18-59 years old.

2.6 Health Risk Assessment

A human health risk assessment is the 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. There are four steps in the Health Risk Assessment. The steps are Hazard Identification, Dose Response Assessment, Exposure Assessment and Risk Characterization (USEPA, 2008).

2.6.1 Hazard Identification

Hazard Identification is the process of determining whether exposure to a stressor can cause an increase in the incidence of specific adverse health effects (e.g., cancer,

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birth defects) and whether the adverse health effect is likely to occur in humans. In the case of chemical stressors, the process examines the available scientific data for a given chemical (or group of chemicals) and develops a weight of evidence to characterize the link between the negative effects and the chemical agent.

Exposure to a stressor may generate many different adverse effects in a human: diseases, formation of tumors, reproductive defects, death, or other effects (USEPA, 2008).

2.6.2 Dose-response assessment

A dose-response relationship describes how the likelihood and severity of adverse health effects (the responses) are related to the amount and condition of exposure to an agent (the dose provided). Although this webpage refers to the "dose-response" relationship, the same principles generally apply for studies where the exposure is to a concentration of the agent (e.g., airborne concentrations applied in inhalation exposure studies), and the resulting information is referred to as the "concentration-response" relationship. The term "exposure-response" relationship may be used to describe either a dose-response or a concentration-response, or other specific exposure conditions.

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Typically, as the dose increases, the measured response also increases. At low doses there may be no response. At some level of dose the responses begin to occur in a small fraction of the study population or at a low probability rate. Both the dose at which response begin to appear and the rate at which it increases given increasing dose can be variable between different pollutants, individuals, exposure routes, etc (USEPA, 2008).

2.6.3 Exposure assessment

EPA defines exposure as 'contact between an agent and the visible exterior of a person (e.g. skin and openings into the body)'. Exposure assessment is the process of measuring or estimating the magnitude, frequency, and duration of human exposure to an agent in the environment, or estimating future exposures for an agent that has not yet been released.

An exposure assessment includes some discussion of the size, nature, and types of human populations exposed to the agent, as well as discussion of the uncertainties in the above information. Exposure can be measured directly, but more commonly is estimated indirectly through consideration of measured concentrations in the environment, consideration of models of chemical transport and fate in the environment, and estimates of human intake over time (USEPA, 2008).

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2.6.4 Risk Characterization

A risk characterization conveys the risk assessor's judgment as to the nature and presence or absence of risks, along with information about how the risk was assessed, where assumptions and uncertainties still exist, and where policy choices will need to be made. Risk characterization takes place in both human health risk assessments and ecological risk assessments.

In practice, each component of the risk assessment (e.g. hazard assessment, dose-response assessment, exposure assessment) has an individual risk characterization written to carry forward the key findings, assumptions, limitations, and uncertainties. The set of these individual risk characterizations provide the information basis to write an integrative risk characterization analysis.

The final, overall risk characterization thus consists of the individual risk characterizations plus an integrative analysis. The overall risk characterization informs the risk manager and others about the rationale behind EPA's approach to conducting the risk assessment - why EPA did what it did to assess the risk (USEPA, 2008)

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CHAPTER 3

METHODOLOGY

3.1 Study Variable

3.1.1 Independent variable

Cadmium concentration in freshwater fish.

3.1.2 Dependent variable

Health risk assessment.

3.2 Study Location

The study was taken place at Kluang, Johor. There were two locations that were involved in this study. The first location was Sembrong River and the respondents were taken from Kampung Sedohok. Meanwhile, the second location was Kahang River and the respondents were taken from Kampung Punjut. Figure 3.1 below shows the map of Kluang in Johor States

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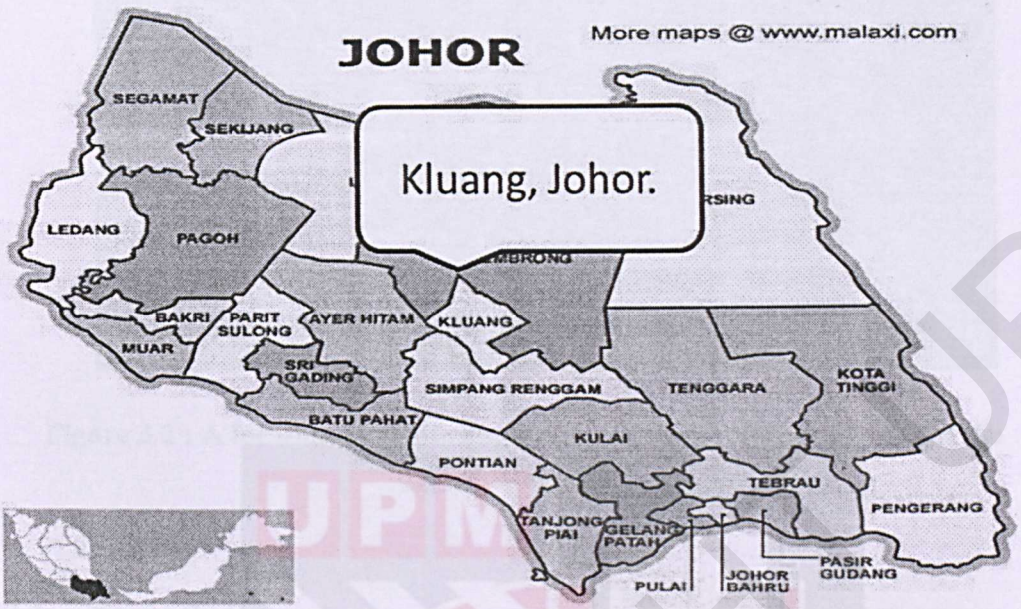


Figure 3.1 : Map of Kluang, Johor.

(Source : Official website of Pejabat Daerah Kluang Johor, 2013)

Usually, fisherman will choose to catch the freshwater fish by using cast nets and they will use boats to catch. Figure 3.2 shows the part of the Sembrong River that the fisherman usually choose in order to catch fishes.

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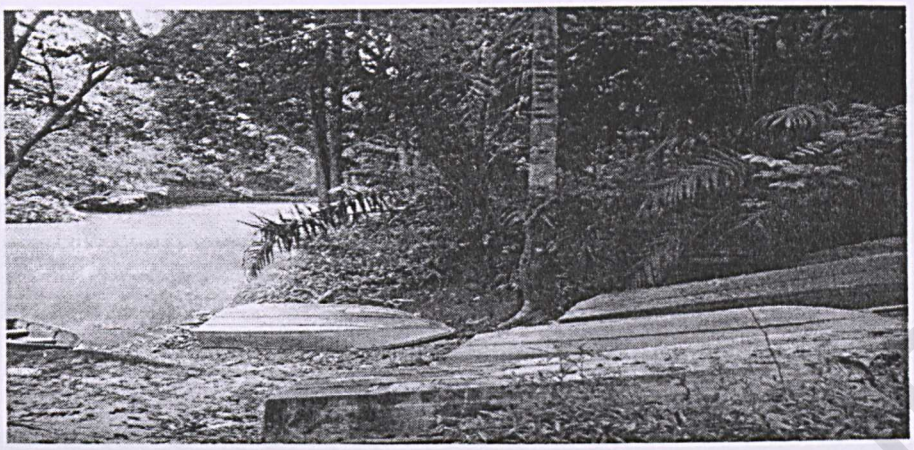


Figure 3.2 : A location at Sembrong River where the fisherman caught the freshwater fish.

3.3 Study Design

The type of study chosen was the cross sectional comparative study. This type of study was chosen because, information is collected from subjects at a single point in time that is from January 2012 until March 2013. This study is very helpful for determining the frequency of risk behavior, which can be very useful in estimating sample sizes for analytic studies.

3.4 Sampling Method

The selection of sampling method for choosing the location was purposive sampling. The chosen location was Kampung Sedohok and Kampung Punjut. The location for the study was purposely chosen as both of the location represent the polluted and less polluted area respectively. Therefore, the sample of freshwater that

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was chosen will be also purposive sampling based on the highest number of frequency intake by the respondents. Then, for the respondents of the both location, the study was using the random sampling method based on the inclusion criteria. Figure 3.3 and Figure 3.4 shows the location of the river with the nearby village (Rogueklamath, 2012).

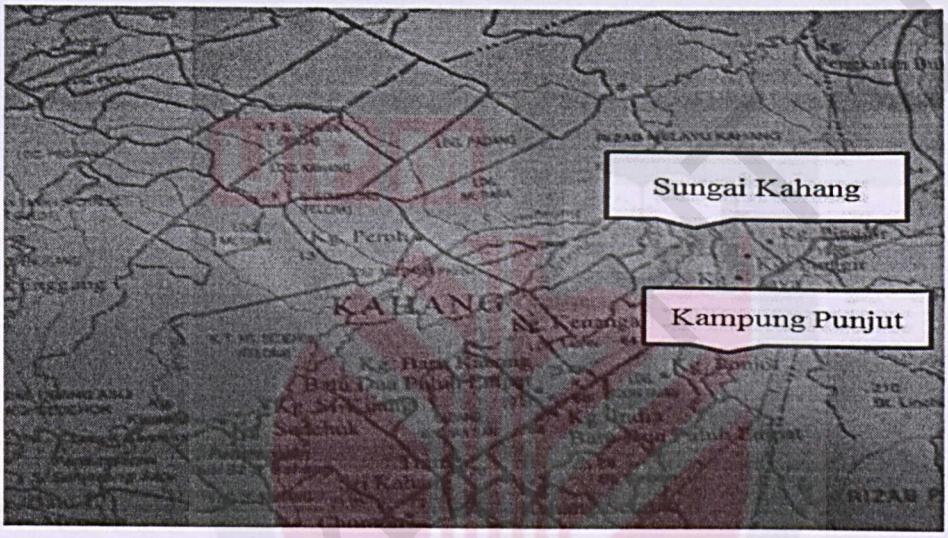


Figure 3.3 : Location of Kahang River that near to Kampung Punjut.
(Source : Pejabat Daerah Kluang Johor, 2013)



Figure 3.4 : Location of Sembrong River that near to Kampung Sedohok.
(Source : Map of Pejabat Daerah Kluang Johor, 2013)

3.5 Study Population

3.5.1 Study Sample

The study samples were *Orang Asli* who lived at the Kampung Sedohok and Kampung Punjut that fulfiled the inclusion criteria for this study. The inclusion criteria include, adult respondents within the range of age of 18 to 59 years old, consumed freshwater fish as their main diet, and do not have any history of chronic disease. The study chose the adult respondents because, it is easier for them to trace and remember the frequency of freshwater fish intake in their diet.

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3.5.2 Sample Size

The number of respondents from Kampung Sedohok and Kampung Punjut were calculated using a formula in order to determine the sample size that need to be involved in the study. Figure 3.5 showed the formula that was used in the study. The formula was used because it was comparing between two groups that were Kampung Sedohok and Kampung Punjut. The formula was based on the mean and standard deviation of higher and lower detection of cadmium in fish based on the previous research by (Fathi *et.al.*, 2012).

The higher standard deviation was added with the lower standard deviation with the power of 2 for each of the standard deviation, then, divided by 2 and the solution was square root to get the final standard deviation. Below is the example of the calculation of final standard deviation.

The mean and standard deviation involved in the previous study of Fathi, *et al,* (2012) is shown as below:

Highest value ; mean : 2.41, standard deviation : 0.15

Lowest value ; mean : 2.32, standard deviation : 0.05

$\delta = \sqrt{[(0.15)^2+(0.05)^2/2]}$

$= 0.1118$

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Therefore, after getting the final standard deviation, it was then replaced to the formula as below:

$$n = \frac{2\sigma^2 [Z_{1-a/2} + Z_{1-B}]^2}{(\mu_1 - \mu_2)^2}$$

Where, n : Sample size

σ : Estimated standard deviation

(assumed to be equal for each group)

μ_1 : Estimated mean (larger)

μ_2 : Estimated mean (smaller)

Figure 3.5 : The formula of sample size that was used in the study.

(Source : Jossey, 2006)

The calculation of the sample size is as follows :

$n=2(0.1118)^2(1.96+0.842)^2/(2.41-2.32)^2$

$=24.2$

$=25$

Therefore, the total respondents for Kampung Sedohok and Kampung Punjut were 25 respondents each.

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3.6 Data Collection and Instrumentation

3.6.1 Data Collection Flow

The data collection flow is important in order to make sure that the study is flowing with systematically and following its target. The data collection flow is can be used as a guide tool in order to tidy up any related planning that need to be done when doing the research so that there is no items can be missing out from the listing.

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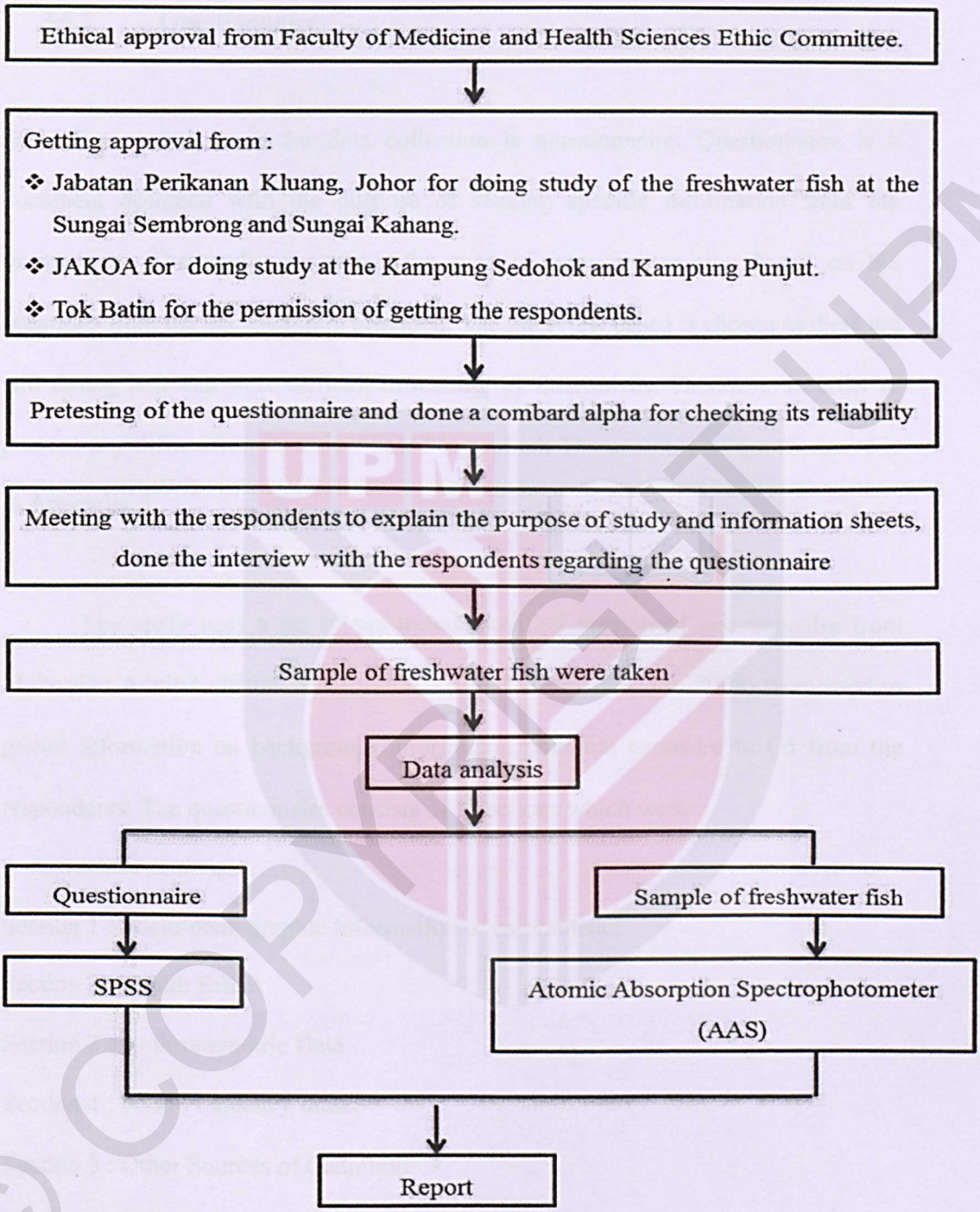


Figure 3.6 : Data collection flow of Cd concentration in freshwater fish and health risk assessment among adults respondents in two areas at Kluang, Johor.

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3.6.2 Questionnaire

One of commonly used for data collection is questionnaire. Questionnaire is a document designed with the purpose of seeking specific information from the respondents. The study was using the type of interview session based on the questions from the questionnaire provided. The interview based is chosen as there are still *Orang Asli* that were difficult in reading by themselves. Therefore, in order to prevent any difficulties, interview based is applied. The example of questionnaire is in Appendix 5.

The study uses a set of pre-tested modified version of questionnaire from Malaysian Adult Nutrition Survey 2002-2003 (Norimah *et. al.*, 2008) were used to gather information on background information potential exposure to Cd from the respondents. The questionnaire consists of 5 sections which were :

Section 1 : Socio-demographic information of respondents

Section 2 : Health Effect

Section 3 : Anthropometric Data

Section 4 : Food Frequency Intake

Section 5 : Other Sources of Cadmium

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Figure 3.7 : The interview session with respondents of Kampung Sedohok

i) Section 1 : Socio-demographic information of respondents

Socio-demographic factors are required in determining the status of the respondents including gender of the respondents, age, race, education level, marital status, monthly income, and religion.

ii) Section 2 : Health Effects

The health conditions that was faced by the respondents were asked. This was to determine whether there was possibility that the respondents have the signs and symptoms regarding the intake of the freshwater fish or any other causes.

iii) Section 3 : Anthropometry Data

The study needed to take the anthropometry data of the respondents. The weight and also the height of the respondents were taken 3 times. Then, the data will be averaged first before starting to calculate the BMI. The BMI was then categorized into underweight (<18.5), normal (18.5-24.99), overweight (≥25.00), and obese (≥30.00). (WHO, 2013)

iv) Section 4 : Food Frequency Intake

The questionnaire regarding the food frequency intake is consists of how frequent the intake or consumption of the freshwater fish among the respondents. The respondents were also have to recall how many times the intake of freshwater fish they might already took in their diet.

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v) **Section 5 : Other Sources of Cadmium**

In this study, the respondents were asked whether they have taken any other sources of cadmium whether through exposure to smoking habits or any other source instead of just consuming freshwater fish. The other sources of Cd included were, referring to the water sources, occupational exposure and lifestyle behaviour through smoking and alcohol consumption among the respondents.

3.6.3 Reagents

Below are the list of reagents used in the study of cadmium concentration in freshwater fish and health risk assessment in two areas at Kluang, Johor.

i) Nitric acid 65%

ii) Deionized water

iii) Distilled water

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3.6.4 Apparatus

Below are the list of apparatus used in the study of cadmium concentration in freshwater fish and health risk assessment in two areas at Kluang, Johor.

i) Atomic Absorption Spectrophotometry (AAS)

ii) Drying oven

iii) Pump and pipette

iv) Beaker

v) Conical flask

vi) Volumetric flask (25ml)

vii) Disposal bottles

viii) Whatman filter paper (0.45µm)

ix) Seca height

x) Seca weight

xi) Weighing balance (for freshwater fish)

xii) Analytical balance

xiii) Standard solution of Cd

xiv) Filter funnel

xv) Sterilize plastic bags

xvi) Alcohol swab

xvii) Hand covering

xviii) Petri dish

xix) Disposable bottles

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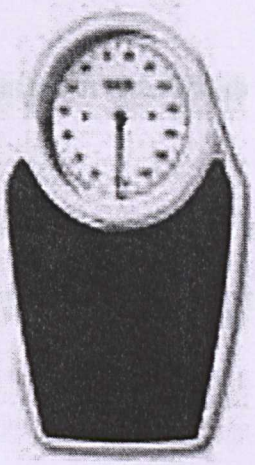


Figure 3.8 : Seca weight to measure the weight of repondents.

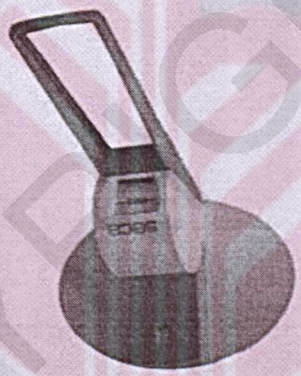


Figure 3.9 : Seca height to measure the height of respondents.

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3.6.5 Cd Concentration in Freshwater Fish

i) Calculation and Preparation of Acid Wash

All the apparatus need to be soaked overnight in the acid wash solution before they were used. While preparing the acid wash, need to always remember to pour the acid into the water.

For preparation of acid wash, the 10% of nitric acid from the 69% of nitric acid was used for the preparation of acid wash digestion. The calculation of acid wash digestion is as follows:

$M_1V_1 = M_2V_2$

$69\ V_1 = 10 \times 4000ml$

$V_1 = (10 \times 4000)/69$

$V_1 = 579.7$ ml make up until 4 liter of water

Therefore, the 10% of nitric acid required is 579.7 ml.

4000ml-579.7ml=3420.3ml

Finally, the remaining deionized water that needed to be make up the solution of acid digestion until 4000ml, was 3420.3 ml.

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ii) Cleaning of Apparatus and Container

Apparatus is cleaning first using distilled water to remove any dirt. Then, cleaning method was adapted from the USEPA Method 1640. All the glassware and plastic were soaked with detergent, then a non metallic brush is used to scrub all the apparatus. Each apparatus is thoroughly rinsed with deionised water.

After that, the apparatus was acid wash by soaked in 10 % nitric acid overnight. The function of acid wash were to make sure that glassware must be physically clean; it must be chemically clean; and in many cases, it must be bacteriologically clean or sterile. all glassware must be absolutely grease-free. (Aldrich, 2013)

After soaking, apparatus is rinsed with distilled water and then with deionised water. Then, glassware is dried using the oven, and plastics were dried in open air.

iii) Freshwater Fish Sample

Three species of frequently consumed freshwater fish were used in this study. The three species that were involved based on the higher number of frequency intake by respondents are *Terubol* species, *Jelawat* species and *Lampam* species. The freshwater species that were taken from both river were the same as to avoid any selection biased occurred throughout the study. All fish samples were purposely

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selected and catch from the points at the two areas at the Kluang, Johor respectively. The freshwater fishes were caught using cast net as shown in Figure 3.10.



Figure 3.10 : The cast net that was used to catch fishes.

The table where the preparation of freshwater fish took place were cleaned first using alcohol swab to prevent any contamination. Then, the freshwater fish were measured for their length, width and weight individually. The freshwater fish samples were washed with deionised water, the inedible parts were removed, and then they were packed in clean zipped polythene bags and transported to the laboratory in an ice-filled polystyrene insulation box. Fish samples were transferred and stored in the laboratory freezer at -20°C to reduce biological deterioration prior to analysis. For each species of freshwater fish, it involved, 3 pieces.

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Table 3.1 : The data of freshwater fishes from Sembrong River.

Sample ID	Species	Average Length (cm)	Average Weight (cm)	Average Width (cm)
FS1	*Terubol*	19.6	140.0	6.4
FS2	*Jelawat*	20.8	120.0	4.6
FS3	*Lampam Jawa*	19.2	120.0	6.7

Table 3.2 : The data of freshwater fishes from Kahang River

Sample ID	Species	Average Length (cm)	Average Weight (g)	Average Width (cm)
FK1	*Terubol*	18.6	130.0	6.2
FK2	*Jelawat*	14.0	90.0	3.8
FK3	*Lampam Jawa*	19.3	160.0	8.0

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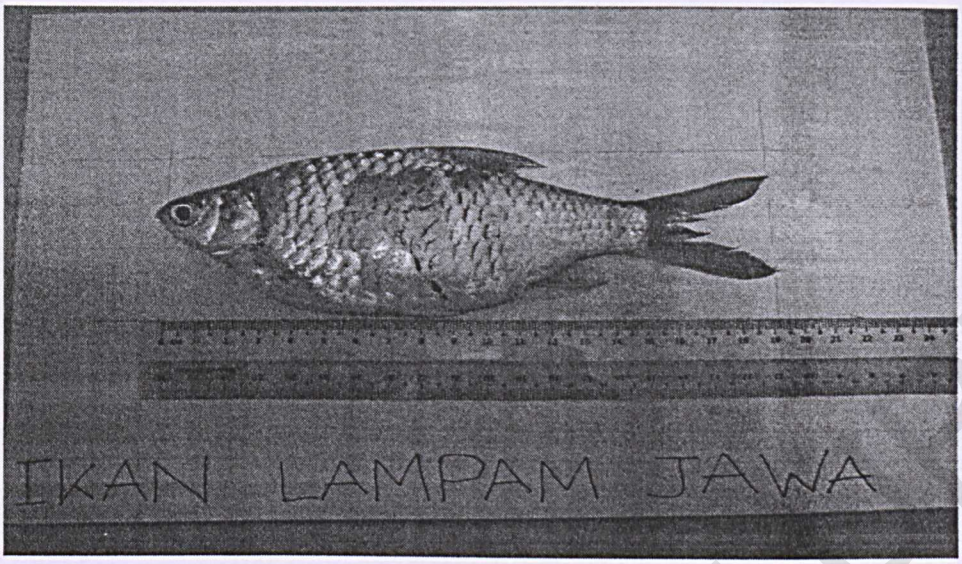


Figure 3.11 : The example of *Lampam Jawa* species.



Figure 3.12 : The example of *Terubol* species

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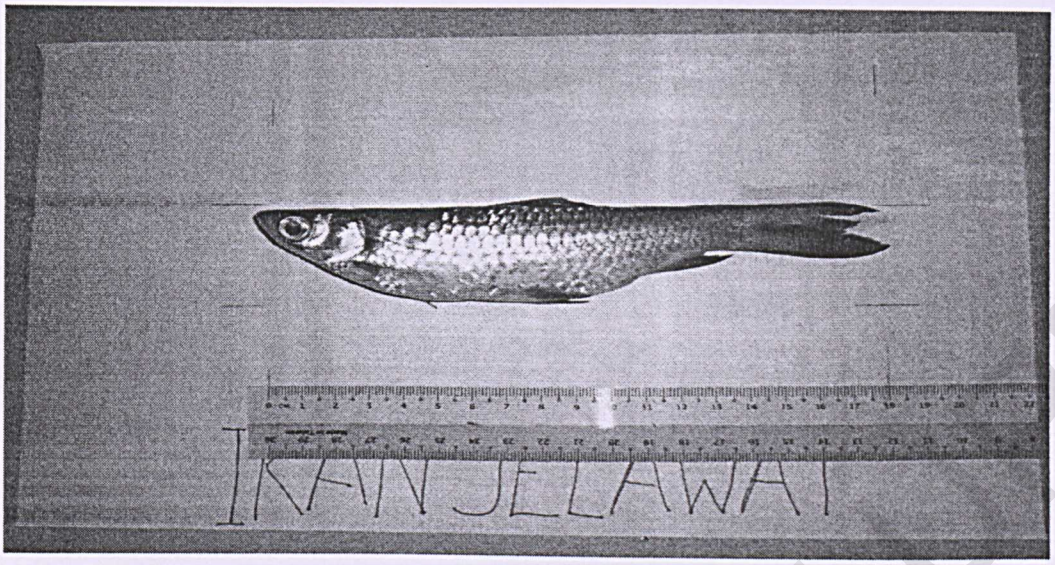


Figure 3.13 : The example of *Jelawat* species.

iv) Dilution of the Concentrated Nitric Acid

The steps of preparation of dilution of concentrated nitric acid are as follows :

$$M_1V_1 = M_2V_2$$

14.4 mol × V_1 = 0.25 mol × 100 ml of distilled water

$$V_1 = 0.25 \times 100/14.4$$

V_1 = 1.736 ml of 65% nitric acid

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Then, added a bit of distilled water into a 100ml volumetric flask. After that, poured 1.736ml of nitric acid into the volumetric flask and then added deionised water until reached the volumetric flask's line.

v) Extraction of Freshwater Fish Sample

The extraction of freshwater fish sample was conducted by using wet acid digestion. This study only take the muscle of the freshwater fish because, the muscle was the part of fish that most people consumed. The scale, and other unclean skin was being removed out from the fish. All the instruments used should be non.metal to avoid any contamination. The muscles were then mixed and grinded by using mortar and pestle so that, it will become softer. Then, the sample were placed in the petri dish. The petri dish with the sample was then dried in the oven for 72 hours at 60-70°C.

The dried sample of freshwater fish from the oven were then again, was grinded by using mortar and pestle to obtain the sample powder. The example of the powder sample were shown as in the Figure 3.14.

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Figure 3.14 : The powdered sample of freshwater fish was grinded by using mortar and pestle.

The sample powder of the freshwater fish was then weighted about 0.5g for each species of freshwater fish.

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Table 3.3 : The weight (g) for each sample (before digestion)

Sample ID	1	2	3
FS1	0.5007	0.5003	0.5004
FS2	0.5001	0.5006	0.5002
FS3	0.5004	0.5000	0.5001
FK1	0.5003	0.5005	0.5002
FK2	0.5002	0.5005	0.5004
FK3	0.5006	0.5004	0.5005

After the samples were weighted, the samples were then added with 5ml of diluted 25M of 65% nitric acid. The samples were kept for 1 day in the fume hood. Then, after that, the prepared samples were then put in the oven for about 3 days with the temperature of 60-80°C until it got clear solution. After digestion, the sample was allowed to cool at room temperature and filtered. The sample was filtered through Whatman No.42 filter paper and then measured again its balance volume. Finally, the sample were made up using 0.25 M nitric acid into 25ml volumetric flask. The samples were then stored in the refrigerator before further analysis.

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Figure 3.15 : The muscles of freshwater fish was being cut.

Table 3.4 : The mean volume of each sample after digestion

Species	Sample ID	Volume (ml)
Terubol	FS1	6.0
Jelawat	FS2	5.9
Lampam	FS3	1.9
Terubol	FK1	3.0
Jelawat	FK2	2.9
Lampam	FK3	2.8

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vi) Preparation of standard solution for Cadmium (Cd)

The standard dilution for cadmium is done for 3 standards ; 0.2 ppm, 0.4 ppm and 0.8 ppm. The stock solution for cadmium is 1000mg/L. Meanwhile, the volume of the mother of the cadmium solution is 100mg/L.

The example of the standard solution for cadmium is as shown in Figure 3.16.

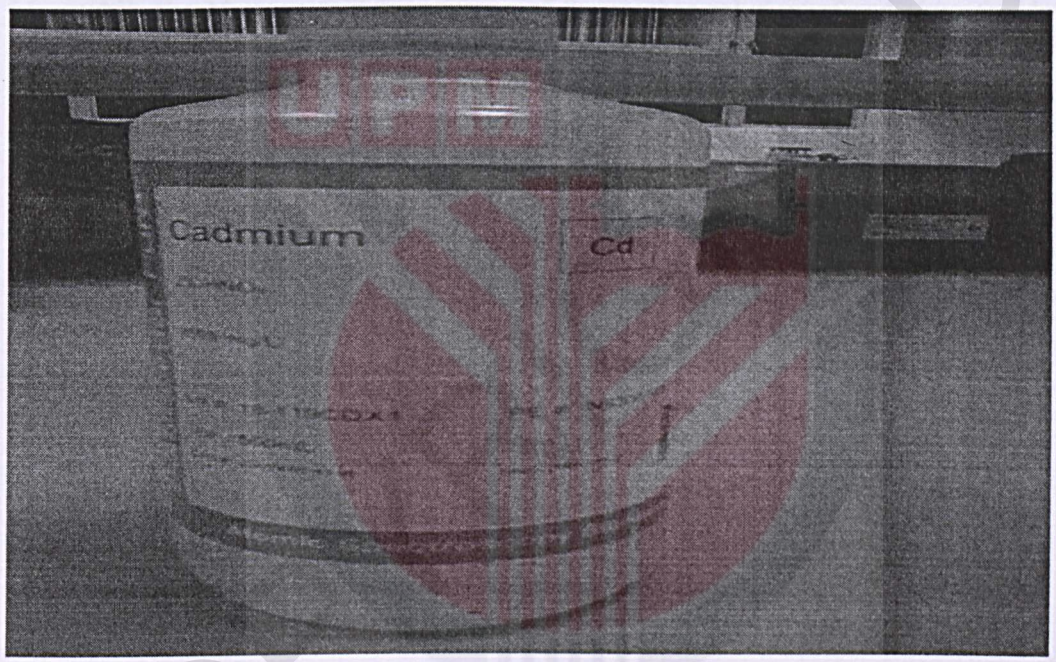


Figure 3.16 : The standard solution of cadmium.

The calculation for each standard is as follows.

$$M_1V_1 = M_2V_2$$

$$1000\ V_1 = 100\ (100)$$

$$V_1 = 10\ ml$$

M_1 represent the volume from the stock solution that is 1000mg/L. Meanwhile the M_2 represent the volume of the mother solution of cadmium that is 100mg/L and V_2 is the volume for the 100ml volumetric flask. Therefore, the volume that need to be taken from the stock solution in order to form a cadmium mother solution is 10ml.

For the calculation of standard of 0.2 ppm is as follows:

$$M_1V_1 = M_2V_2$$

$$100\ V_1 = 0.2\ (100)$$

$$V_1 = 0.2\ ml$$

For the calculation of standard of 0.4 ppm is as follows:

$$M_1V_1 = M_2V_2$$

$$100\ V_1 = 0.4\ (100)$$

$$V_1 = 0.4\ ml$$

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For the calculation of standard of 0.8 ppm is as follows:

$$M_1V_1 = M_2V_2$$

$$100\ V_1 = 0.8\ (100)$$

$$V_1 = 0.8\ ml$$

Therefore, each of the standard; 0.2 ml, 0.4 ml and 0.8 ml need to be taken from the mother of cadmium standard and then, made up with deionised water until 100 ml of volumetric flask. The prepared standard is then transferred in a tube for further analysis by using AAS.

Figure 3.17 shows the example of the mother solution of cadmium that was being create from the standard of cadmium.

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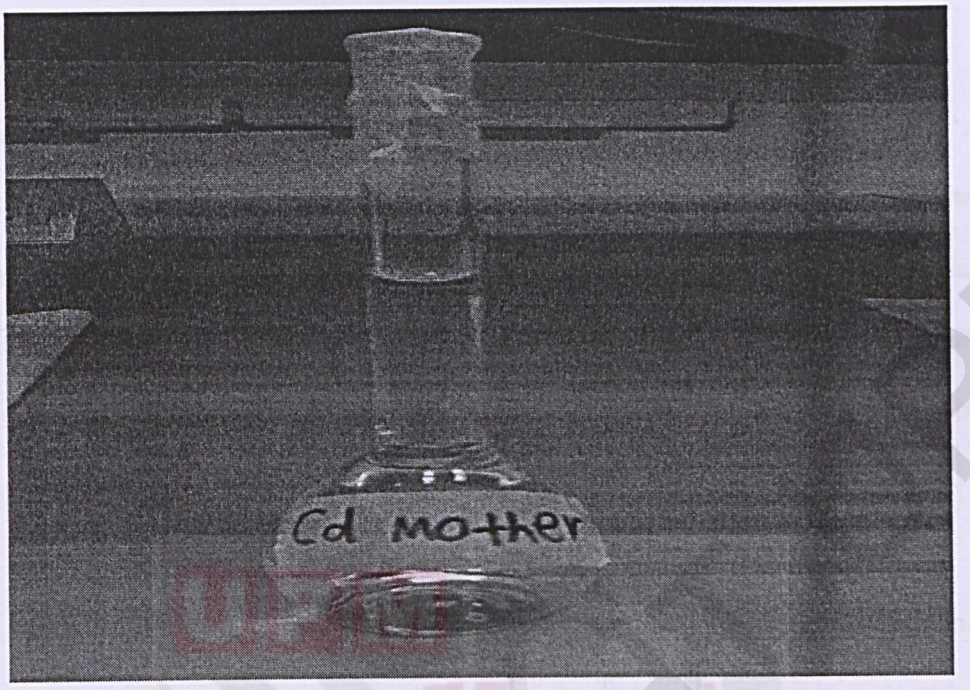


Figure 3.17 : The mother solution of cadmium.

vii) Preparation of blank sample

The blank sample for the analysis by using AAS. The blank sample involve the dilution of nitric acid and deionised water. The use of blank ensures that all errors of measurements that may be introduced into absorption spectrum from cuvette material, solvent, temperature fluctuations, gases in the atmosphere are taken into account.

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viii) Cd concentration determination

Cadmium is determined by atomic absorption spectrometry in conjunction with a graphite furnace containing a graphite platform. A sample is placed on the graphite platform and a matrix modifier is added. The sample is then evaporated to dryness, charred, and atomized using high-temperature ramping. The absorption signal generated during atomization is recorded and compared with standards (Caslab, 2012).

Figure 3.18. shows the samples of freshwater fish that were ready for the determination of its cadmium concentration in the AAS. There were 6 samples involved, which is 3 samples from Kampung Sedohok and another 3 samples from Kampung Punjut. Each samples were labelled.

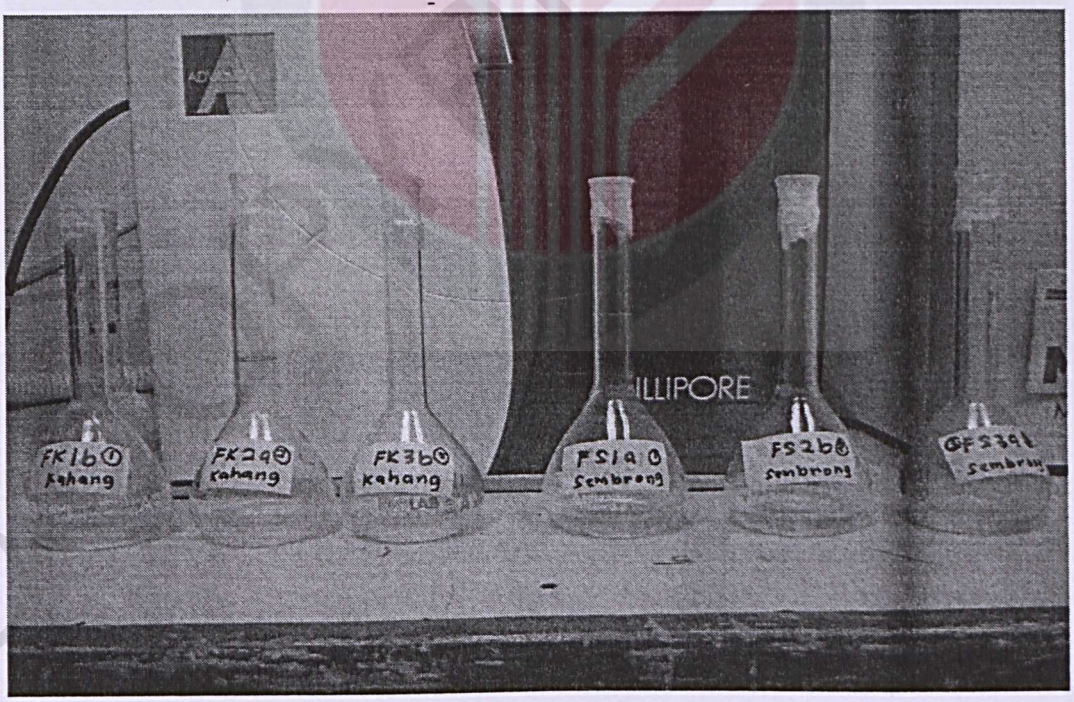


Figure 3.18 : Samples for analysis of freshwater fishes.

The samples of the freshwater fish from the volumetric flask were then transferred to the tube in order to put into the AAS's equipment as shown in Figure 3.19.

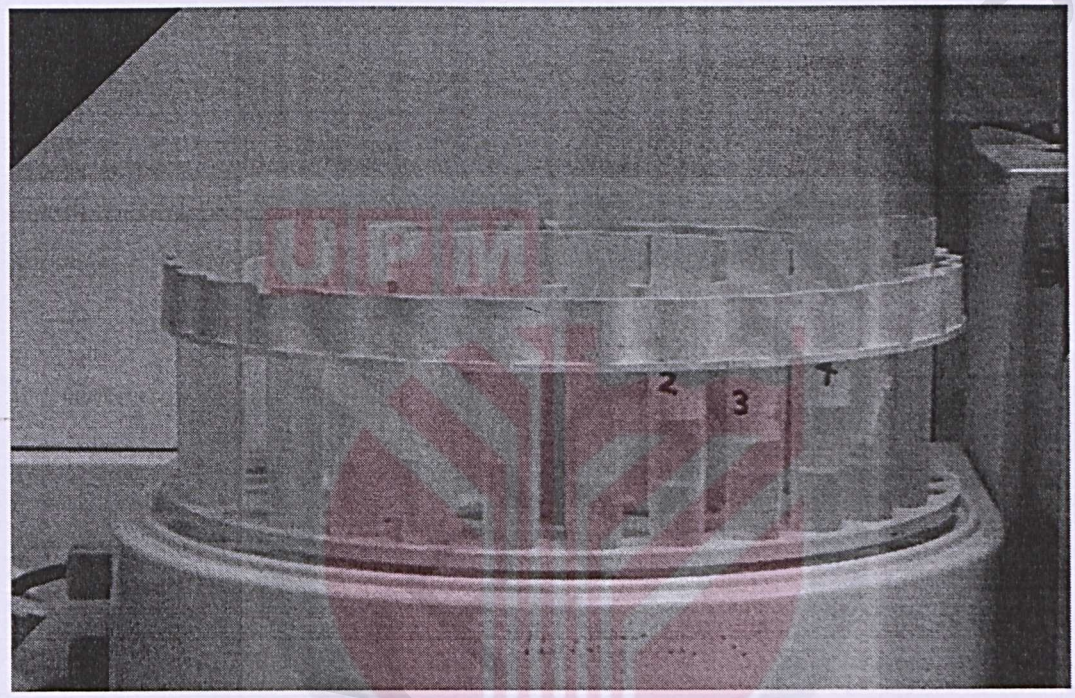


Figure 3.19 : The tube that contain samples.

Cd was determined by the calibration method with one blank and three (3) standard solutions of 0.2 ppm, 0.4 ppm and 0.8 ppm.

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ix) Calculation for Cd concentration from AAS

The result from the AAS was calculated as according to the formulae calculation stated below:

The cadmium concentration in freshwater fish sample (mg/L) is calculated as below:

$$= (A \times B \times \frac{C}{W})$$

Where :

A = The Cd concentration in the extract (mg/L)

B = Dilution factor

C = The volume of extract (ml)

W = Weight of the sample (0.5g)

The result for the calculation of the final cadmium concentration were used to determine the health risk assessment of respondents.

3.6.5 Health Risk Assessment

The Health Risk Assessment (HRA) was evaluated from analysing of the questionnaires regarding the daily intake of the freshwater fish. Therefore, the calculation of the lifetime cancer risk (LCR) and hazard quotient (HQ) was

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calculated in order to determine the carcinogenic health risk and non-carcinogenic health risk of respondents respectively.

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

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

Whereby:

ADD / LADD	:	Intake of cadmium (mg/kg/day)
C	:	Total of cadmium concentration in canned fish (mg/kg)
IR	:	Ingestion rate of canned fish (kg/day)
ED	:	Exposure duration (year)
EF	:	Exposure frequency (day/year) [EF = (F (day/year) x ED) / AT]
BW	:	Body weight (kg)
*AT		Averaging time (days)

Value for AT :

*ADD	ED x 365 day-year
*LADD	Life time equal to 25,550 days based on lifetime exposure for 70 years.

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Estimation of risk for non-carcinogens:

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

Where,

HQ : Non-cancer Hazard Index of a health effect from intake of cadmium

ADD : Average daily dose (mg kg–1 day–1)

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

*RfD Cd – 5.0 x 10-4 per kg body weight

(USEPA, 2008).

Estimation of risk for carcinogen:

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

Where:

LCR : Upper limit cancer risk

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

q* : Cancer potency factor, also known as slope factor (mg kg–1day–1)

Cd cancer slope factor (CSF) (mg kg–1 day–1) = 6.1

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A HQ of less than 1 was considered as acceptable as this means that the ADD was below the RfD. Meanwhile, for Lifetime Excess Cancer Risk is acceptable in the range of 10^{-6} to 10^{-4}. The summary of HQ and LCR is as below:

HQ	:	(<1)	Acceptable
		(>1)	Not acceptable
LCR	:	(<10^{-6})	Clearly acceptable
		(10^{-6} to 10^{-4})	Acceptable
		(>10^{-4})	Clearly unacceptable

3.7 Quality Control

3.7.1 Standard Operating Procedure (SOP)

Standard Operating Procedures (SOP) are in place in many laboratories. These are documented procedures that are carried out regularly as part of the routine and could cover many different aspects of the work. For example, how tests are carried out, the procedures for handling the chemicals or samples involved and the disposal of samples or waste. This could also cover the way different pieces of equipment must be used and maintained, including the correct solutions for use in them. In conclusion, the SOP means it was to minimize analytical errors.

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The analysis of freshwater fish sample using Atomic Absorption Spectroscopy follow the standard operating procedure as given by the manufacturer and daily performance report of the AAS were run.

3.7.2 Glassware Apparatus

All glassware used in this study were washed with detergent, soaked in nitric acid and rinsed with deionised water to minimize and prohibit external metal contamination. The acid wash was done for all apparatus before used.

3.7.3 Pre-test

A very important part of the questionnaire contruction process is its piloting, known as pretesting. This involves testing research instrument in conditions as similar as possible to the research, but not in order to report results but rather to check for glitches in wording of questions, lack of clarity of instructions etc. - in fact, anything that could impede the instrument's ability to collect data in an economical and systematic fashion (IMS, 2003).

Pre-test of questionnaire was conducted for testing the reliability of the questionnaire. The Cronbach's alpha was done in order to determine the level of reliability of questionnaire to the respondents by using reliability test in the SPSS. Otherwise, one of the most commonly used indicators of internal consistency is Cronbach's alpha coefficient. The result from cronbach's alpha was 0.86. In this

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study, the pre-test was done at the Kampung Tanah Runtuh. This village is chosen because, it shares the same characteristics of study respondents and thus, it is representative for the actual respondents of Kampung Sedohok and Kampung Punjut. About 10% from the sample size was included in this pre-test that was 6 respondents.



Figure 3.20 : The pre-test questionnaire that was done at nearby village that is Kampung Tanah Runtuh

3.8 Statistical analysis

All the data was analyzed by using the Statistical Packages for Social Sciences (SPSS) version 21.

For the first objective is to determine the socio-demography of the respondents. The statistical analysis involved is descriptive analysis. The element that were tested including age, household income, BMI, household person, gender, religion, marital status, job sector and education.

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For the second objective is to compare the frequency intake of freshwater fish in two areas. The statistical analysis involved is chi-square. The chi-square was chosen because, this objective was about comparing between two group of dichotomous group.

For the third objective is to compare the cadmium concentration of freshwater fish between two areas. The statistical analysis involved is Mann-Whitney U test. The test was chosen because, this objective was about comparing between two group. The normality test was not normally distributed.

For the fourth objective is to compare health risk assessment of the respondents between two areas. The statistical analysis involved is Mann-Whitney U test. The test was chosen because, this objective was about comparing between two group. The normality test was not normally distributed.

The last statistical analysis is the relationship between the frequency intake of freshwater fish and health risk is using Spearman test to obtain the correlation among those factor and in fact, it is for not normally distributed.

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3.9 Ethical concern

Before the study was conducted, ethical concern are taken into consideration before any study can be proceed. Approval were obtained from the University Research Ethics Committee, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia (UPM) before the study was carried out.

Beside that, as this study involved the *Orang Asli* of the related village, the approval from the Jabaran Kemajuan Hal Ehwal Orang Asli, Ampang and as well as Jabatan Kemajuan Hal Ehwal Orang Asli, Kluang Johor must be obtained before taken any of the respondents of *Orang Asli.*

Otherwise, approval from the head of both village that is Kampung Sedohok and Kampung Punjut and as well as approval from respondents also must be obtained first before carried out the study. Explaination about the purpose and procedures of the study may given to the respondents.

Participation consent form was given to each respondents who participate in this study. In another word, the written consent will be obtained from the respondents prior to the study. Otherwise, the information of the respondents especially their identities were remained confidential. It is only using the code number instead of name, in order to maintain the confidentiality of the respondents. Furthermore, their information were not appeared in any parts of the study.

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CHAPTER 4

RESULTS

4.1 Response rate

In this study, twenty five respondents were needed to accomplish this study. However, thirty respondents from each village were recruited. All of them agreed to participate in this study and the response rate was a hundred and twenty percent (120%). This excellent response rate was achieved due to the good collaboration between the organization of JAKOA, Department of Fisheries, head of village (Tok Batin of Kampung Sedohok and Kampung Punjut) and respondents.

4.2 Socio-demographic information of respondents

Table 4.1a and Table 4.1b shows the socio-demographic information of respondents from both Kampung Sedohok and Kampung Punjut. The first objective of this study is to determine the socio-demographic data of the respondents of both

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Kampung Sedohok and Kampung Punjut. The data include, age, gender, race, religion, marital status, education level, job sector, BMI, household income, and number of household.

The majority of gender for both Kampung Sedohok and Kampung Punjut was male respondents that is 63.3% and 56.7% respectively. Meanwhile for the female respondents, Kampung Sedohok was 36.7%, while Kampung Punjut was 43.4%. In term of the age, majority of respondents from Kampung Sedohok was in the range of age of 26-30 that is 26%, while Kampung Punjut was in the range of age 18-25 that is 30%. Moreover, 29 out of 30 respondents of Kampung Sedohok was animisme while only one respondent was Muslim. Meanwhile, 100% of the respondents from Kampung Punjut was animisme. In terms of marital status, most of them were already married in which at Kampung Sedohok, 86.7% while at Kampung Punjut was 80%. For job sector, 50% of respondents from Kampung Sedohok do not working, while 50% of respondents from Kampung Punjut was a self-employed.

For education level of respondents, both of Kampung Sedohok and Kampung Punjut were studied at the primary level education that was 63.3% and 40% respectively. For the household income, both of Kampung Sedohok and Kampung Punjut have the same amount of income that was 63.3% for income of less than RM760. Meanwhile for income of more than RM760, was 36.7%.

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For BMI, both respondents of Kampung Sedohok and Kampung Punjut was in the range of normal that was 56.7% and 46.7% respectively. For the number of household, most of both Kampung Sedohok and Kampung Punjut have more than 5 person in a house, that is 86.7% and 60% respectively.

Table 4.1a: Socio-demographic information of the respondents.

Variable	Kampung Sedohok (N=30)			Kampung Punjut (N=30)		
	Freq.	%	Mean ± S.D	Freq.	%	Mean ± S.D
Age			32.4 ± 8.4			33.9 ± 11.7
18 – 25	7	23.3		9	30.0	
26 – 30	8	26.7		3	10.0	
31 – 35	5	16.7		7	23.3	
36 – 40	6	20.0		4	13.3	
41 – 45	1	3.3		1	3.3	
46 – 50	2	6.7		2	6.7	
51 – 55	0	0.0		2	6.7	
56 – 59	1	3.3		2	6.7	
Household income			551.6 ± 368.0			725.0 ± 482.5
≤ 760	19	63.3		19	63.3	
> 760	11	36.7		11	36.7	
BMI			23.3 ± 5.4			28.1 ± 13.6
<18.5 (Underweight)	4	13.3		3	10.0	
18.5-24.9 (Normal)	17	56.7		14	46.7	
25-29.9 (Overweight)	6	20.0		5	16.7	
30≥ (Obese)	3	10.0		8	26.7	
Household person			4.6 ± 2.2			5.5 ± 2.4
≤ 5	26	86.7		18	60.0	
> 5	4	13.3		12	40.0	

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Table 4.1b: Socio-demographic information of the respondents.

Variable	Kampung Sedohok (N=30)		Kampung Punjut (N=30)	
	Freq.	%	Freq.	%
Gender				
Male	19	63.3	17	56.7
Female	11	37.6	13	43.3
Religion				
Muslim	1	3.3	0	0.0
Animisme	29	96.7	30	100.0
Marital Status				
Single	4	3.3	6	20.0
Married	26	86.7	24	80.0
Job sector				
Employed	4	13.3	1	3.3
Self- employed	11	36.7	15	50.0
Not working/ Others	15	50.0	14	46.7
Education				
Not Attend School	7	23.3	11	36.7
Kindergarden /Primary	19	63.3	12	40.0
Secondary /Higher	4	13.4	7	23.3

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4.3 Frequency intake of freshwater fish.

Figure 4.1 shows the frequency intake of freshwater fish of respondents from both Kampung Sedohok and Kampung Punjut. It exhibits that about 60% of the respondents from Kampung Punjut eat freshwater fish in their daily meal compared to Kampung Sedohok that is 66%. Otherwise, for the 3 to 5 times per week consumption, Kampung Punjut and Kampung Sedohok were 10% and 20% respectively. Otherwise, for the once a week consumption, Kampung Punjut and Kampung Sedohok were 10% and 7% respectively. Furthermore, for the 2 to 3 times per month consumption, Kampung Punjut and Kampung Sedohok were 10% and 7% respectively. Moreover, for once in a month consumption, 7% of the respondents from Kampung Punjut was involved while there is none from Kampung Sedohok that eat freshwater fish once in a month. However, there is still respondents from Kampung Punjut that was seldom consume the freshwater fish in their diet, compared to Kampung Sedohok that is none of the respondents that was seldomly eaten freshwater fish.

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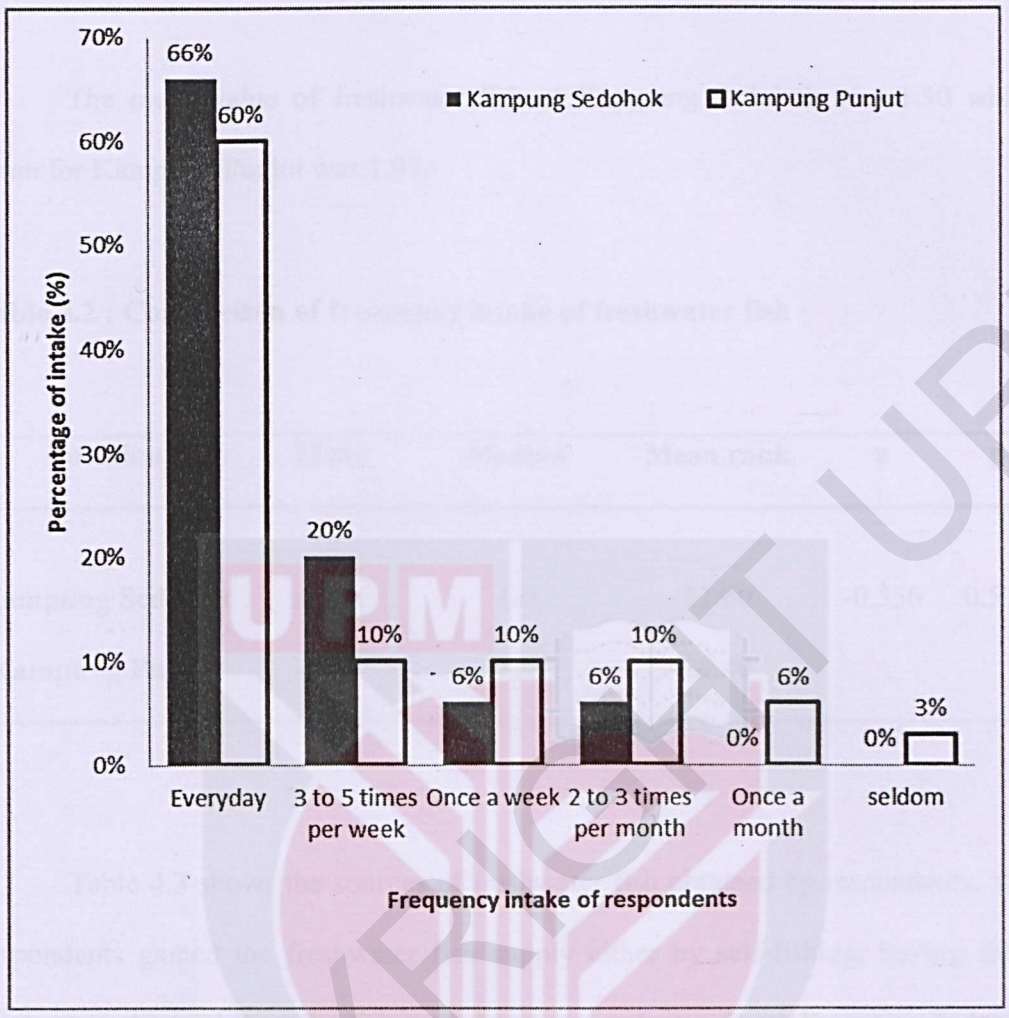


Figure 4.1 : The frequency intake of freshwater fish among respondents.

4.4 Comparison of frequency intake of freshwater fish of the respondents.

Table 4.2 below show the frequency intake of the freshwater fish among respondents. The test involved is Mann-Whitney U test. Based on the result obtained, it is clearly seen that, there is no significance difference between the frequency intake of freshwater fish among respondents from both Kampung Sedohok and Kampung Punjut. The significant value is at $p<0.05$.

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The mean value of freshwater fish at Kampung Sedohok was 1.50 while mean for Kampung Punjut was 1.97.

Table 4.2 : Comparison of frequency intake of freshwater fish

Location	Mean	Median	Mean rank	z	p
Kampung Sedohok	1.50	1.0	31.60	-0.556	0.578
Kampung Punjut	1.97	1.0	29.40		

Table 4.3 shows the sources of freshwater fish obtained by respondents. The respondents gained the freshwater fish supply either by self-fishing, buying from markets or obtained from the fish cage. Most respondents of both Kampung Sedohok and Kampung Punjut were obtained their sources of freshwater fish by self-fishing that was 83% and 60% respectively, followed by markets that was 17% and 36% respectively. However, there is none of respondents from Kampung Sedohok that obtained their freshwater fish through the fish cage, but for Kampung Punjut, there was 3.3% of the respondents that obtained the freshwater fish through fish cage.

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Table 4.3 : The source of freshwater fish for both Kampung Sedohok and Kampung Punjut

Sources of Fresh water fish	Kampung Sedohok (n=30)		Kampung Punjut (n=30)	
	Freq.	%	Freq.	%
Self-fishing	25	83	18	60
Market	5	17	11	36.7
Fish cage	0	0	1	3.3

4.5 Other possible exposure to cadmium

There are other sources of cadmium exposure other than through food contamination. It includes contamination in drinking water, occupational exposure, smoking habit and alcohol consumption. Table 4.4 shows the water sources utilized by respondents. All of the respondents (100%) from Kampung Punjut was using the tap water. For Kampung Sedohok, only one respondent that used tap water that was 3.3%. Otherwise, the rest of 96.7% of the respondents from Kampung Punjut was using the well water.

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For the smoking habits, 53.3% of respondents from Kampung Sedohok was smoking, meanwhile 46.7% of respondents for Kampung Punjut was not smoking. For the occupational exposure, 43.3% of respondents from Kampung Sedohok was exposed to the occupational exposure compared to only 30% of the respondents from Kampung Punjut that was exposed to occupational exposure. For alcohol consumption, 23.3 % of the respondents from Kampung Sedohok drink alcohol, while 26.7% of respondents from Kampung Punjut was not drinking alcohol.

Table 4.4 : Other possible sources of cadmium

	Kampung Sedohok (n=30)		Kampung Punjut (n=30)	
Variables	**Freq.**	**%**	**Freq.**	**%**
Water sources				
Tap water	1	3.3	30	100.0
Well water	29	96.7	0	0.0
Smoking habits				
Yes	16	53.3	14	46.7
No	14	46.7	16	53.3
Occupational exposure				
Yes	13	43.3	9	30.0
No	17	56.7	21	70.0
Alcohol consumption				
Yes	7	23.3	8	26.7
No	23	76.7	22	73.3

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Table 4.5 and Table 4.6 shows the prevalence and mean frequency of the top ten daily consumed foods which are potential source of cadmium among respondents from Kampung Sedohok and Kampung Punjut.

In Table 4.5, generally, 90% of the respondents from Kampung Sedohok consumed sugar everyday. The other nine food items eaten daily but by a smaller proportion of the population ranged from sugar, soy sauce, shrimp paste, chilli sauce, leafy vegetables, anchovy, salad, oyster sauce, fish cake and cabbage. All of these food items are mostly consumed about once to twice per day.

Otherwise, in Table 4.6, generally, 83% of the respondents from Kampung Punjut consumed sugar everyday. The other nine food items eaten daily but by a smaller proportion of the population ranged from sugar, soy sauce, anchovy, shrimp paste, leafy vegetables, long bean vegetables, chilli sauce, cabbage, oyster sauce and salad. All of these food items are mostly consumed about once to twice per day.

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Table 4.5 : Prevalence and mean frequency of the top ten daily consumed food among respondents from Kampung Sedohok.

Variables	Kampung Sedohok (n=30)	
	Prevalence (%)	Mean frequency per day
Sugar	90	2.00
Soy Sauce	77	2.00
Shrimp Paste	57	3.18
Chili sauce	53	2.00
Leafy vegetable	50	2.13
Anchovy	47	2.36
Salad (ulam)	37	2.18
Oyster sauce	33	1.90
Fish cake	30	1.50
Cabbage	20	2.00

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Table 4.6 : Prevalence and mean frequency of the top ten daily consumed food among respondents from Kampung Punjut.

Variables	Kampung Punjut (n=30)	
	Prevalence (%)	Mean frequency per day
Sugar	83	2.10
Soy Sauce	70	1.36
Anchovy	50	2.13
Shrimp paste	47	3.14
Leafy vegetable	43	2.07
Long bean vegetable	43	1.92
Chili sauce	37	1.45
Cabbage	27	2.25
Oyster sauce	23	1.57
Salad (ulam)	23	1.85

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Table 4.7 and Table 4.8 shows the prevalence and mean frequency of the top ten weekly consumed foods among respondents from Kampung Sedohok and Kampung Punjut.

In Table 4.7, generally, 60% of the respondents from Kampung Sedohok frequently consumed long leafy vegetable weekly with the mean of 1.83 per weekly. The other nine food items eaten weekly but by a smaller proportion of the population ranged from long leafy vegetables, canned fish, *keropok lekor*, baby corn, tofu, salad, anchovy, ground nuts, peanut and also wet prawn. All of these food items are mostly consumed about once to twice per week.

Otherwise, in Table 4.8 shows the prevalence and mean frequency of the top ten food items consumed weekly by the adult population for Kampung Punjut. Generally, 57% of the respondents from Kampung Punjut frequently consumed long leafy vegetable weekly with the mean of 1.53 per weekly. The other nine food items eaten weekly but by a smaller proportion of the population ranged from fish cake, bean sprouts, tempe, peanut, *keropok lekor,* cabbage, tapioca, anchovy, canned fish, and cuttle fish, All of these food items are mostly consumed about once to twice per week.

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Table 4.7 : Prevalence and mean frequency of the top ten weekly consumed food among respondents from Kampung Sedohok.

Variables	Kampung Sedohok (n=30)	
	Prevalence (%)	Mean frequency per weekly
Long bean vegetable	60	1.83
Canned fish	53	1.31
Kerepok lekor	53	1.43
Baby corn	50	1.70
Tofu	50	1.73
Salad (ulam)	47	2.07
Anchovy	43	2.71
Ground nut	43	1.85
Peanut	43	1.62
Prawn (wet)	40	2.08

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Table 4.8 : Prevalence and mean frequency of the top ten food items consumed weekly by the adult populations for Kampung Punjut

Variables	Kampung Punjut (n=30)	
	Prevalence (%)	Mean frequency per weekly
Fish cake	57	1.53
Bean sprout	47	1.93
Tempe	43	1.86
Peanut	43	1.46
Keropok Lekor	40	1.33
Cabbage	40	1.33
Tapioca	37	1.36
Anchovy	33	1.90
Canned fish	33	1.50
Cuttle fish (wet)	33	2.08

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4.6 Cadmium concentration in freshwater fish

Figure 4.2 shows the cadmium concentration detected in six (6) samples of freshwater fish in two areas at Kluang, Johor that was three (3) from Sembrong River (Kampung Sedohok) and another three (3) from Kahang River (Kampung Punjut). The samples were analysed by using AAS after being digested with wet acid digestion. All the samples were triplicates and reported in mg/L. This three (3) samples from each village indicated the mostly consumed species of freshwater fish among the respondents of the area.

Table 4.9 shows the parameters used in order to convert raw data from reading of AAS to actual reading Cd concentration. After the conversion, the study shows that, for the Kampung Sedohok, the mean of Cd concentration in the freshwater fish of sample 1 is 4.92 mg/kg, sample 2 is 7.29 mg/kg, and sample 3 is 6.77 mg/kg. For the Kampung Punjut, the mean of Cd concentration in freshwater fish of sample 1 is 4.68 mg/kg, sample 2 is 4.69 mg/kg, and sample 3 is 4.98 mg/kg.

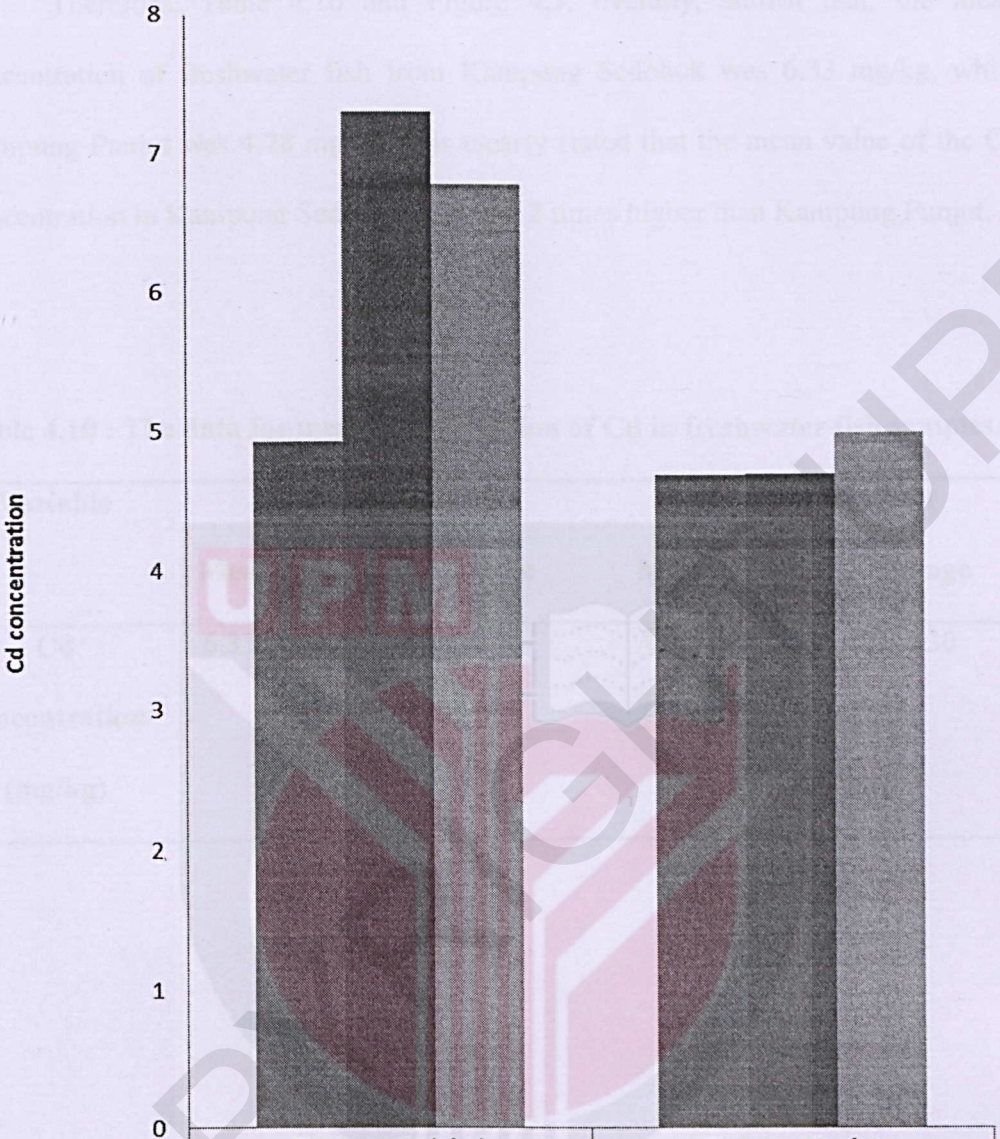
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Table 4.9 : Parameters used to convert raw data of AAS to actual reading of Cd concentration.

Sample	A (mg/L)	B	C (ml)	W (g)	Final concentration of Cd (mg/kg)
FS1	0.0100	41	6.0	0.5	4.92
FS2	0.0147	42	5.9	0.5	7.29
FS3	0.0136	131	1.9	0.5	6.77
FK1	0.0094	83	3.0	0.5	4.68
FK2	0.0094	86	2.9	0.5	4.69
FK3	0.0100	89	2.8	0.5	4.98

A-Concentration of Cd in the extract
B-Dilution factor
C-Volume of extract
W-Weight of sample

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	Kampung Sedohok	Kampung Punjut
Terbol	4.92	4.68
Jelawat	7.29	4.69
Lampam Jawa	6.77	4.98

Figure 4.2 : Cd concentration in freshwater fish of both Kampung Sedohok and Kampung Punjut.

Therefore, Table 4.10 and Figure 4.3, overally, shown that, the mean concentration of freshwater fish from Kampung Sedohok was 6.33 mg/kg, while Kampung Punjut was 4.78 mg/kg. It is clearly stated that the mean value of the Cd concentration in Kampung Sedohok is nearly 2 times higher than Kampung Punjut.

Table 4.10 : The data for mean concentration of Cd in freshwater fish samples.

Variable	Kampung Sedohok		Kampung Punjut	
	Mean ± SD	Range	Mean ± SD	Range
Cd concentration (mg/kg)	6.33 ± 1.25	2.37	4.78 ± 0.17	0.30

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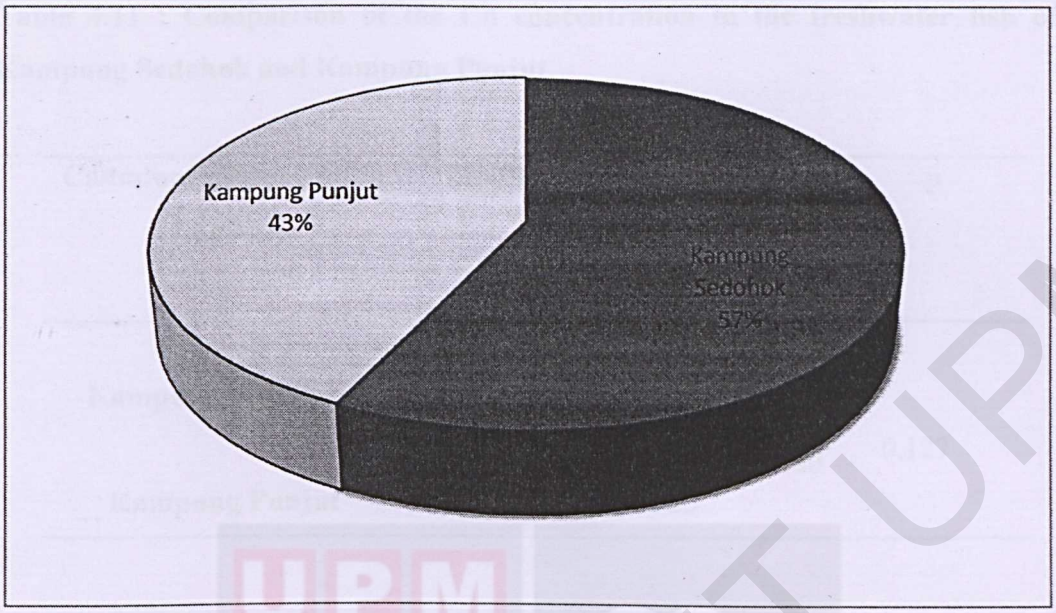


Figure 4.3 : The pie-chart of the mean cadmium concentration.

4.7 Comparison of Cd concentration in freshwater fish in the 2 areas.

Table 4.11 shows the comparison of the Cd concentration in the freshwater fish of Kampung Sedohok and Kampung Punjut. Mann-Whitney U test was used to determine whether there is a significant difference of the cadmium concentration in freshwater fish in the two areas. It was found that there is no significant difference between the cadmium concentration in freshwater fish between Kampung Sedohok and Kampung Punjut. The p value is significant at $p<0.05$..

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Table 4.11 : Comparison of the Cd concentration in the freshwater fish of Kampung Sedohok and Kampung Punjut.

Cadmium concentration (mg/kg)	Median	Mean rank	z	p
Kampung Sedohok	6.770	4.67	1.00	0.127
Kampung Punjut	4.690	2.33		

4.8 Health risk encountered by respondents from cadmium exposure through consumption of freshwater fish.

Table 4.12 shows the results obtained on the mean value of the Average Daily Dose (ADD), Lifetime Cancer Risk (LADD), Hazard Quotient (HQ) and Lifetime Cancer Risk (LCR) of the respondents of both areas.

For Kampung Sedohok, the mean of ADD is 1.775×10^{-6} while Kampung Punjut is $53.6\times10^{-6.}$ Otherwise, for the value of LADD, the mean for Kampung Sedohok is 7.975×10^{-6} and the mean of LADD for Kampung Punjut is $22.3\times10^{-6.}$ This result shows that the mean value of ADD and LADD for Kampung Sedohok is higher than Kampung Punjut.

For the lifetime cancer risk (LCR) indicated for health risk of carcinogenic health effects encountered by respondents. The LCR presented in 3 categories which

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were clearly acceptable, acceptable and clearly unacceptable. For Kampung Sedohok and Kampung Punjut, there was 100% respondents had clearly acceptable risk with mean of 0.486×10^{-5} and 1.347×10^{-6} respectively.

The hazard quotient (HQ) indicated for health risk of non-carcinogenic health effects encountered by respondents. The HQ presented in 2 categories which were acceptable and clearly unacceptable. For Kampung Sedohok and Kampung Punjut, there was 100% respondents have acceptable risk with mean of 2.8×10^{-3} and 1.07×10^{-3} respectively.

However, it is seen that the value of mean of HQ and LCR for Kampung Sedohok is slightly higher compared to Kampung Punjut.

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Table 4.12 : Mean and standard deviation of the value ADD, LADD, HQ, and LCR.

Variable	Kampung Sedohok (n=30)	Kampung Punjut (n=30)
	Mean±SD	Mean±SD
ADD	$1.775\times10^{-6} \pm 3.266\times10^{-6}$	$53.6\times10^{-6} \pm 1.787\times10^{-6}$
LADD	$7.975\times10^{-6} \pm 0.152\times10^{-6}$	$22.3\times10^{-6} \pm 74.3\times10^{-6}$
HQ		
Acceptable (<1) Not acceptable (>1)	$2.8\times10^{-3} \pm 5.14\times10^{-3}$	$1.07\times10^{-3} \pm 3.57\times10^{-3}$
LCR		
Clearly acceptable ($<10^{-6}$) Acceptable (10^{-6} to 10^{-4}) Clearly unacceptable ($>10^{-4}$)	$0.486\times10^{-5} \pm 0.926\times10^{-5}$	$1.347\times10^{-6} \pm 4.535\times10^{-6}$

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4.9 Comparison of HQ for non-carcinogenic health effects and LCR for carcinogenic health effects.

Table 4.13 shows the comparison of the health risk of HQ and LCR of the respondents between two areas. Mann-Whitney U Test was used to determine whether there is a significant difference between the health risk of respondents of the two areas. It was found that there is a significant difference between the health risk that is HQ and LCR of respondents between both Kampung Sedohok and Kampung Punjut. The significant value is at $p<0.05$.

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Table 4.13 : Comparison of hazard quotient (HQ) and Lifetime cancer risk (LCR) of the respondents between the two areas.

Variables	Median	IQR	Mean rank	Z	p-value
Hazard quotient (HQ)					
Kampung Sedohok	3.954×10^{-4}	3.7×10^{-3}	38.03	-3.351	0.01*
Kampung Punjut	2.004×10^{-4}	1.842×10^{-4}	22.97		
Lifetime Cancer Risk (LCR)					
Kampung Sedohok	5.591×10^{-6}	5.246×10^{-5}	44.2	-6.098	<0.0001*
Kampung Punjut	2.5411×10^{-7}	2.482×10^{-7}	16.8		

*significant at $p<0.05$

z=Mann-Whitney U

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4.10 Relationship between frequency intake of freshwater fish and health risk assessment in the two areas.

Table 4.14 shows the relationship between the frequency intake of freshwater fish and lifetime cancer risk (LCR) for carcinogenic health effects and Hazard Quotient (HQ) for non-carcinogenic health effects of respondents in the two areas. The test involved in order to obtain the correlation is Spearman' rho. Based on the results of lifetime cancer risk (LCR), it was found that for Kampung Sedohok, there is a significant relationship between frequency intake of freshwater fish and lifetime cancer risk (LCR). Otherwise, the results from Kampung Punjut stated that there is no significant relationship between frequency intake of freshwater fish and lifetime cancer risk (LCR).

Furthermore, based on the results of hazard quotient (HQ), it was found that for Kampung Sedohok, there is a significant relationship between frequency intake of freshwater fish and hazard quotient (HQ). Otherwise, the results from Kampung Punjut stated that there is no significant relationship between frequency intake of freshwater fish and hazard quotient (HQ).

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Table 4.14 : Spearman's rho test between freshwater fish frequency intake and health risk LCR and HQ.

Location	LCR	
	r	P
Kampung Sedohok	0.810	<0.0001*
Kampung Punjut	-0.147	0.439
	HQ	
Kampung Sedohok	0.8	<0.0001*
Kampung Punjut	-0.2	0.289

*significant at $p<0.05$

r=correlation coefficient

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CHAPTER 5

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Discussion

This study was done to determine the cadmium concentration of freshwater fish in the two areas at Kluang, Johor. The areas involved Kampung Sedohok as the representation for polluted river (Sembrong River) and Kampung Punjut as the representation for less polluted river (Kahang River). The respondents from both areas were *Orang Asli.* The facilities at the both areas, were quite limited at Kampung Sedohok compared to Kampung Punjut as the water source at Kampung Sedohok was mostly from well water and Kampung Punjut was from tap water. However the other facilities such as main hall and school is quite similar. The total population for Kampung Sedohok was 190 while Kampung Punjut was 240. The self-fishing is one of the activity that was done for both respondents in order to get the source of freshwater fish for consuming purpose.

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Otherwise, the way to access to the Kampung Sedohok as shown in the Figure 5.1 was quite difficult as it was situated further from the main road and the condition of the road access was not quite maintain compared to Kampung Punjut. Thus, it was taken quite a longer time to reach the location of Kampung Sedohok compared to Kampung Punjut that was 45 minutes and 15 minutes respectively.



Figure 5.1 : The access to the Kampung Sedohok.

Meanwhile, for Kampung Punjut, the road access was well maintained and the location was not far from the main road, thus easy to access. Moreover, the population at Kampung Punjut was also seen to have a better quality of life compared to Kampung Sedohok as most of the population has car at home.

As the conclusion, the both villages were chosen because, both village were recommended by the Department of Fisheries, Kluang Johor. Otherwise, both of this

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village has the more population than the others and also easier to get the permission from Tok Batin. Moreover, the respondents from the both villages were confirm to obtain and consumed the freshwater fish from the related river.

5.1.1 Socio-demography of the respondents.

The socio-demography of the respondents were obtained first before proceed to the research. The information obtained was important in order to make a confirmation that the respondents are suitable to be involved in the study. The sampling for the respondents was based on random sampling but based on inclusion criteria. In this study, both of the areas, represent almost similar characteristics in terms of socio-demography information. Therefore, they were suitable to be compared for cadmium concentration based on their intake and not being biased by different level of socio economic factors.

Furthermore, the similar number of household income for both Kampung Sedohok and Kampung Punjut indicated that in terms of salary, both of them share the same level. In fact, this less value of RM760 indicate majority of respondents in both villages fall into poverty group. The RM760 is the poverty line index for rural of Peninsular Malaysia (PLI, 2013)

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5.1.2 Comparison of frequency intake of freshwater fish

The frequency intake of the freshwater fish in both areas shows almost similar number of intake of the freshwater fish in their daily diet. This frequency intake of freshwater fish in study areas were supported with the statement of Jabatan Kemajuan Hal Ehwal Orang Asli (JAKOA), that stated, the culture of *Orang Asli* will tend to consume freshwater fish as their main diet instead of marine fish. Otherwise, from the informal interview with the Tok Batin of Kampung Sedohok, it was found that most of the *Orang Asli* catched fish for their domestic consumption, mostly by woman community.

Moreover, the fish was also easily to find compared to other aquatic life such as prawn that usually depending on its climate factors and come out within a specific season. The lower quality of life of *Orang Asli* also was one of the factors that they have to consume freshwater fish that is easily to get for free at the related river.

Otherwise, the chosen of respondents from both Kampung Sedohok and Kampung Punjut were suitable for the purpose of this study due to the similar frequency intake of the freshwater fish in their diet.

Moreover, the respondents from both river will tend to consume the near source freshwater fish at their related area.

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The frequency intake of freshwater fish was important to be known in order to determine the ADD and LADD of each respondent. The information on ADD is needed for the estimation of respondents' non-carcinogenic health risk. USEPA Integrated Risk Information System (IRIS) defined ADD as the dose rate averaged over a pathway-specific period of exposure expressed as a daily dose on a per-unit-body-weight basis and Lifetime Average Daily Dose (LADD) is the estimated dose to an individual averaged over a lifetime of 70 years; used in assessments of carcinogenic risk (USEPA, 2012).

5.1.3 Other possible sources of cadmium

The exposure to cadmium might come from other types of exposure. An experimental studies was done indicate that, cadmium was more readily found in the free ionic form in water, while in food, the cadmium ion generally exists in a complex with a variety of ligands, including proteins such as metallothionein. Experimental studies in animals have generally used soluble salts of cadmium (such as cadmium chloride) for food, drinking water, and gavage exposures. The toxicological properties of the cadmium ion do not appear to depend on the counter ion, although absorption may be significantly affected by protein complexes (ATSDR, 2007).

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Based on the results from the top ten daily and weekly consumed other foods by the respondents, there are also types of foods that possibly to contained cadmium. The foods are cabbage, peanut, canned fish, cuttlefish, long bean vegetables and salad (ulam). On the year of 2009, CAP president S.M. Mohamed Idris said random tests conducted on samples of cuttlefish found that the amount of cadmium exceeded the safety level (Manjit, 2009).

5.1.4 Cadmium concentration of freshwater fish.

The study found that, the cadmium concentration in fish collected from Kampung Sedohok is nearly 2 times higher than Kampung Punjut with the ratio of 3:2. The higher level of cadmium detected at the Kampung Sedohok compared to Kampung Punjut is maybe due to the availability of the source of cadmium at the area. Along Sembrong River, there are more industrialization available compared to Kahang River. The example of industrialization are oil palm factory, rubber tree, gas of methane project, compost fertilizer, treatment plant, Kimberly Clark and Huggies.

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Figure 5.2 : The wood manufacturing near to Sembrong River.



Figure 5.3 : The unknown substances found at Sembrong River.



Figure 5.4 : The oil palm manufacturing nearby to Sembrong River and Kampung Sedohok.



Figure 5.5 : The compost fertilizer in the area of Kampung Sedohok and Sembrong River.

A study was done indicated that, the results of many field studies of metal accumulation in fish living in polluted waters show that considerable amounts of various metals may be deposited in fish tissues without causing mortality. Various metals are accumulated in fish body in different amounts. These differences result from different affinity of metals to fish tissues, different uptake, deposition and excretion rates (Wordpress, 2011).

Moreover, it is difficult to compare the heavy metal concentration even between the same species because of different feeding habitat, difference in aquatic environment, level of water pollution, growing rate of the species and other factors (Wordpress, 2011).

5.1.5 Comparison of Cd concentration in freshwater fish in the 2 areas.

Generally, the mean concentration of Cd in Kampung Sedohok were higher compared to Kampung Punjut. This clearly indicates the influence of aquatic environments as well as surrounding human activities at specific point of time may contribute to accumulation of heavy metals in fishery products. Accumulation of heavy metals in the aquatic environments have been associated with urban runoff, sewage treatment plants, industrial effluents and wastes, mining operations, boating activities, domestic garbage dumps and agricultural fungicide runoff (Sivaperumal *et al*., 2003).

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Otherwise, it was seen that, there was an unknown substances situated near to Sembrong River, as shown in Figure 5.6.

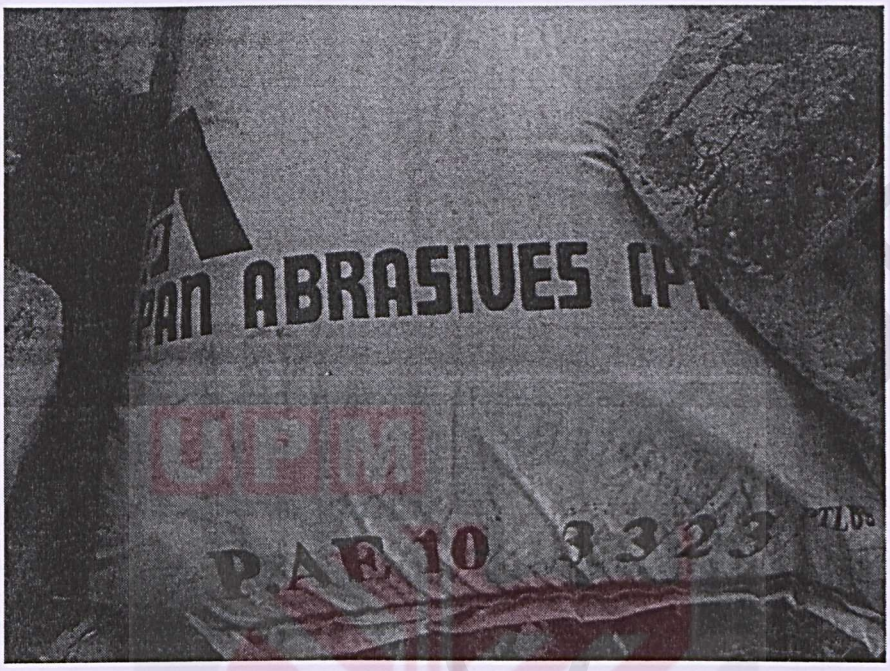


Figure 5.6 : The abrasive substances found near to Sembrong River.

In this study for the comparison of Cd concentration in freshwater fish in the 2 areas involved the Mann-Whitney U test. The p-value is more than 0.05 that is p=0.127. Therefore, that is why it is concluded that there is no significant differences of cadmium concentration between Kampung Sedohok and Kampung Punjut.

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5.1.6 Health risk encountered by respondents from cadmium exposure through consumption of freshwater fish.

The value for HQ and LCR of respondents from Kampung Sedohok is higher compared to Kampung Punjut. This means that respondents in Kampung Sedohok face more health risk by consuming this polluted freshwater fish as compared to respondents of Kampung Punjut.

Therefore, the higher cadmium in the freshwater fish, may lead to higher health risk for those who consume. The detected cadmium in freshwater fish may gives various effects to consumers. There was a research done by a team of researchers from the DOE Office of Science's Argonne National Laboratory (2003) has demonstrated for the first time that cadmium releases calcium from bone within hours of exposure, even at concentrations below current Occupational Safety and Health Administration (OSHA) standards. In humans, this response might lead to the bone disease osteoporosis. Therefore, cadmium may accelerates net bone loss.

5.1.7 Comparison of HQ for non-carcinogenic health effects and LCR for carcinogenic health effects.

Respondents in Kampung Sedohok face more health risk by consuming polluted fish as compared to respondents from Kampung Punjut. In the long term, it may posed

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some serious health problems as exposure to cadmium may caused kidney damage, liver damage, bone defect and brain damage.

This study found that all of the respondents fall under clearly acceptable risks towards the carcinogenic and non-carcinogenic health risk. It might be due to the low level of cadmium concentration in the freshwater fish of the study area and also the value of ADD and LADD of the respondents are unlikely to cause adverse health effects.

There is a study conducted by the Department of Health and Human Services (DHHS) and the International Agency for Research on Cancer (IARC) have determined that cadmium and cadmium compounds are human carcinogens. The EPA determined that cadmium is a probable human carcinogen (ATSDR, 2007)

Lung cancer has been found in some studies of workers exposed to cadmium in the air and studies of rats that breathed in cadmium. The U.S. Department of Health and Human Services (DHHS) has determined that cadmium and cadmium compounds are known human carcinogens. The International Agency for Research on Cancer (IARC) has determined that cadmium is carcinogenic to humans. The EPA has determined that cadmium is a probable human carcinogen (ATSDR, 2007).

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5.1.8 Relationship between frequency intake of freshwater fish and health risk assessment in the two areas.

Based on the results of lifetime cancer risk (LCR), it was found that for Kampung Sedohok, there was significant relationship between frequency intake of freshwater fish and lifetime cancer risk (LCR). Otherwise, the results from Kampung Punjut stated that there was no significant relationship between frequency intake of freshwater fish and lifetime cancer risk (LCR).

This was because, the respondents from Kampung Sedohok was detected with the higher mean concentration of cadmium in fish compared to Kampung Punjut. Otherwise, this finding of Kampung Sedohok, was supported by one of the research that has been done about the heavy metal contamination of soils resulting from mining and smelting that causing major concern due to the potential risk involved. The study was designed to investigate the heavy metal (Cu, Zn, Pb and Cd) concentrations in soils and food crops and estimated the potential health risks of metals to humans via consumption of polluted food crops grown at four villages around the Dabaoshan mine, South China. The heavy metal concentrations in paddy and garden soils exceeded the maximum allowable concentrations for Chinese agricultural soil (Sivaperumal *et., al*., 2007)

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The industrialization of compost fertiliser near to Kampung Sedohok also may lead to the higher level of accumulation of cadmium in fish. Otherwise, based on the GuildFord rule of Thumb, the correlation coefficient of 0.8 for hazard quotient of Kampung Sedohok and 0.810 for lifetime cancer risk of Kampung Sedohok is in the range of 0.7 to 0.9 that is strong relationship.

Due to high frequency of freshwater fish consumption of relatively high concentration of cadmium in fish from Kampung Sedohok, therefore a strong significant relationship was found between fish consumption and risk assessment.

Furthermore, the findings from the significant relationship between frequency intake of freshwater fish and health risk assessment among respondents can be proven with the research done by Al-Khateeb and Leilah, (2005) that stated, fish has been found to be excellent indicator for the heavy metal contamination level in aquatic system because it occupies different food chain and thus this may lead to excessive accumulation which cause problems to human, animal, and plants.

Otherwise, the results from Kampung Sedohok showed significant relationships between frequency intake and the health risk assessment because accumulation of cadmium in the body through continuously small quantity intake may cause health effects such as kidney disease.

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The statement was supported by the previous study where cadmium is primarily toxic to the kidney, especially to the proximal tubular cells where it accumulates over time and may cause renal dysfunction. After prolonged or high exposure the tubular damage may progress to decreased glomerular filtration rate, and eventually to renal failure (ATSDR, 2008). Besides that, long term exposure to high dose cadmium causes itai-itai disease. Its syndrome principally consists of a painful skeletal condition resulting from weak and deformed bones. The disease is characterized initially by complaints of spinal and leg bone pain, and an increasingly waddling gait due to bone deformities. These symptoms can persist and typically progress for several years, until the patient is eventually unable to walk and become bedridden. The clinical symptoms then progress rapidly, with eventually severe debilitating pain, multiple bone fractures from even mild traumas such as coughing, severe skeletal deformities, anemia and severe kidney problems such as impaired tubular and glomerular function and generalized osteomalacia and osteoporosis that result in multiple bone fractures and leading to death (Inaba *et al.,* 2005).

5.1.9 Study strength

The study of fishes is really suitable for this kind of specific time's research as fishes do always available compared to other aquatic life for example, prawns that only occur in a specific season.

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Otherwise, it is a good choice to take fish as the research sample than othe aquatic life as fish is seen as at the top of the aquatic food chain and may accumulate large amount of heavy metals from its environment (Mansour and Sidky, 2002). Moreover, a previous study also, stated that, Tilapia fish have a greater capacity for metal bioaccumulation than tiger prawn (Mokhtar *et.al.,* 2009)

Otherwise, the adults respondents are chosen because it is easier for them to trace and remember the frequency of freshwater fish intake in their diet. Moreover, the health risk assessment is done for this study may help in indicating the risks that may faced by respondents as the exposure to the cadmium.

Moreover, according to En. Taib, Chief of State Officer of Department of Fisheries stated that, this is the first study regarding the cadmium concentration in freshwater fish and as well as doing the health risk assessment among *Orang Asli* at at Kluang.

5.1.10 Study limitation

The limitation of this study was the seasonal condition. The sample of freshwater fish cannot be taken in the condition of flooding or rainy season. Therefore, the suitable timeframe need to be consider for the sampling purpose. On the other hand,

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this study only focusing on the freshwater fish in river. Thus, maybe for the further study, can investigate the comparison of the freshwater fish in cage and in the river can be done. The freshwater fish in cage usually is given a pellet as their food source compared to freshwater fish at the river that is eaten whatever components they have in the water.

Otherwise, the study sample is also quite small. For further study, maybe can increase the sample size taken from the respondents. In this study, the small sample size was obtained because, we go to both Kampung Sedohok and Kampung Punjut at the daytime and usually, most of *Orang Asli* are working by self-fishing at that time. For us, it is quite difficult in order to go for the night time as because of the purpose of safety and as well as the condition of road access to the both village that is quite dark in the night time. Thus, we have to go out from the village before the night time.

5.2 Conclusion

The study indicated that the fish from the polluted Sembrong River shows a slightly higher in cadmium concentration compared to less polluted Kahang River. Otherwise, the health risk (HQ) and (LCR) of respondents are also slightly higher in polluted Sembrong River compared to less polluted Kahang River. Therefore, there is a significant relationship between the frequency intake of consuming the contaminated fish and health risk assessment of respondents. Moreover, the higher

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class of river and the increase in the manufacturing plants along the Sembrong River may lead to the higher number of cadmium concentration and also the health risk assessment of the respondents. Thus further research into the potential effects of cadmium exposure is warranted.

5.3 Recommendation

There are several improvement should be done or can be done for further of study for a better findings. It is recommended that resercher to take along the biological sample instead of only take the sample of fishes in order to confirm the detection of cadmium. The biological sample is taken in order to ensure and strengthen the proof about the level of cadmium contained in the fishes. If the cadmium is accumulated to longer time in the body, blood is the best indicator in order to test it.

Otherwise, to make it more valuable if taking blood as the biological sample, researcher can make a differentiation in terms of level of cadmium within the content of the blood. For example, the level of cadmium in the whole blood, plasma, or red blood cell. From there, researcher could know which part is the most highest concentration of heavy metal can be found.

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Moreover, it is recommended for the respondents to practise a good nutritional intake of the foods that may help in flushing out the accumulation of cadmium in the body. The vitamin C for example can be consumed by the respondents because the vitamin C is one of the example of supplement that enable and help to combine those heavy metal in the body and thus excrete the heavy metal out of the body easily. The heavy metal in this study is cadmium. In this study as my study is regarding the *Orang Asli,* maybe as a researcher, we can approach the Jabatan Hal Ehwal Orang Asli (JAKOA) that is the responsible body that taking care of the welfare of *Orang Asli,* may in order giving them for free of that kind of supplement

Moreover, the periodic surveillance also need to be done if the concentration of cadmium is confirm to be exceeded for the Malaysia permitted level that is 1 ppm in the aquatic fish. The population also cannot consume the freshwater fish for a while until the concentration of cadmium can be controlled. Otherwise, if the level of the cadmium is more increasing in the future, the related responsible body for example the District Health Office may give order to stop the operation of the manufacturing.

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APPENDIX 1 : ETHICAL COMMITTEE CONSENT LETTER

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JAWATANKUASA ETIKA PENYELIDIKAN PERUBATAN
UNIVERSITI PUTRA MALAYSIA

PROPOSAL PENYELIDIKAN (PERMOHONAN BARU PRASISWAZAH)

Bil	No. Rujukan	Jab.	Tajuk	Penyelidik	Penyelia	Human Body Involvement	Ulasan Ahli		Catatan
							Lulus	Pending	
3	JKPP(U)_Oct(12)32	JKPP	Cadmium Concentration in Freshwater Fish and Health Risk Assessment Among Adults Between Two Areas at Kluang, Johor	Hilaliyah Mohkhtar@Mokhtar	Dr. Saliza Mohd Elias	1) Questionnaire/ Interviews 2) Physiological Measurements 6) Use of Body fluids (e.g. blood)	/		(1) must mention who will collect the blood and how much (vol. of blood taken) (2) Must not have name & address of respondent but use code of respondent - the name & address come together an and the sheet.

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APPENDIX 2 : APPROVAL LETTER FROM JAKOA, AMPANG

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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.

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

Ruj. Kami : JAKOA.PP.30.032 Jld.26 (39)
Tarikh : 27/8 R' Awal 1434H
Februari 2013

- Faks diterima 13.2.13 -

PEJABAT KEMAJUAN ORANG ASLI
DITERIMA
19 FEB 2013
DAERAH KLUANG

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 **"Kepekatan Kadmium di Dalam Ikan Air Tawar dan Penilaian Risiko Kesihatan di Dua Kawasan di 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	:	**Hilaliyah bt Mohkhtar@Mokhtar**

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:		
TARIKH:		
PEGAWAI KOA DAERAH	TINDAKAN	
PEMBANGUNAN SOSIO EKONOMI	MAKLUMAT	
PERANCANGAN & PENYELIDIKAN	EDARAN	
KESIHATAN		
MINDA & PENDIDIKAN	SIMPANAN	

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APPENDIX 3 :
INFORMATION SHEET

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

PENGENALAN

Pencemaran logam-logam seperti kadmium tersebut dapat mempengaruhi dan menyebabkan penyakit pada konsumen, karena di dalam tubuh unsur yang berlebihan akan mengalami detoksifikasi sehingga membahayakan manusia. Logam berat umumnya bersifat racun terhadap makhluk hidup, walaupun beberapa diantaranya diperlukan dalam jumlah kecil. Melalui berbagai perantara, seperti udara, makanan, maupun air yang terkontaminasi oleh logam berat, logam tersebut dapat terdistribusi ke bagian tubuh manusia dan sebagian akan terakumulasikan. Jika keadaan ini berlangsung terus menerus, dalam jangka waktu lama dapat mencapai jumlah yang membahayakan kesehatan manusia. Kajian ini mengkaji mengenai kandungan kadmium dalam ikan air tawar dan penilaian risiko kesihatan di kalangan orang dewasa di dua kawasan iaitu Kampung Sedohok dan Kampung Pucur di Kluang, Johor.

APAKAH YANG PERLU ANDA LAKUKAN?

Responden perlu menjawab soalan berkenaan dengan kajian yang akan disoal. Pengkaji akan menggunakan kaedah menemuramah responden. Responden juga perlu menandatangani borang kerelaan untuk memberi kerjasama kepada pengkaji sepanjang kajian dijalankan. Jika persetujuan telah dilakukan, responden telah menjadi sebahagian ahli daripada kajian ini. 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 pengamal perubatan yang bertauliah yang diiktiraf oleh Kementerian Kesihatan Malaysia (KKM). Pengamal perubatan tersebut juga mempunyai pengalaman dalam pengambilan darah menggunakan kaedah 'Venipuncture'.

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.

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APAKAH FAEDAH MENYERTAI KAJIAN INI?

a) KEPADA ANDA SEBAGAI PESERTA?

Responden akan dapat membantu penyelidik membuat penemuan terhadap kajian mereka. Selain itu, responden dapat membantu penyelidik mendapatkan bilangan kouota responden yag ingin dicapai. Selain itu, 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 memperolehi data untuk kajian. Penyelidik juga akan meningkatkan dan memperluaskan informasi berkenaan tajuk untuk kajian ini. Selain itu, penyelidik juga berkebolehan untuk meningkatkan kemahiran komunikasi apabila berkomunikasi dengan responden.

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, pengamal 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?

Semestinya menjadi sebahagian daripada etika kajian untuk merahsiakan maklumat peribadi para responden. 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.

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SIAPA YANG SAYA PERLU HUBUNGI SEKIRANYA SAYA MEMPUNYAI SOALAN TAMBAHAN SEMASA MENGIKUTI PENYELIDIKAN INI?

Jika terdapat sebarang pertanyaan mengenai kajian ini atau masalah-masalah lain, responden boleh menghubungi penyelidik dan juga penyelia penyelidik.

Penyelidik: Hilaliyah Binti Mohkhtar@Mokhtar

Bacelor Sains (Kesihatan Persekitaran dan Pekerjaan)

Jabatan Kesihatan Persekitaran dan Pekerjaan,

Fakulti Perubatan dan Sains Kesihatan

Universiti Putra Malaysia

43400 UPM, Serdang

Selangor Darul Ehsan.

Tel: 013-4637768

E-mel: hilaliyah_mokhtar@yahoo.com

Penyelia: Dr. Saliza Binti Mohd Elias

Jabatan Kesihatan Persekitaran dan Pekerjaan,

Fakulti Perubatan dan Sains Kesihatan

Universiti Putra Malaysia

43400 UPM, Serdang

Selangor Darul Ehsan.

Tel : 603-89472402

E-mel : saliza@medic.upm.edu.my

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APPENDIX 4 :
CONSENT LETTER

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

PENYELIDIK :

ANA MARDHIAH BINTI MARZUKI
CHRISTOPHER GEORGE
HILALIYAH BINTI MOKHTAR
NOOR AZIANTI BINTI ZAKARIA

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 identity 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. Kad Pengenalan :.....

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

Tarikh .....

Tandatangan .....
(Penyelidik)

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APPENDIX 5 : QUESTIONNAIRES

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ID Peserta :

JABATAN KESIHATAN PERSEKITARAN DAN PEKERJAAN
FAKULTI PERUBATAN DAN SAINS KESIHATAN
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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.

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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

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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
.....

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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)*

$$\text{BMI} = \frac{\text{Berat (kg)}}{\text{Tiggi x Tinggi (m2)}}$$

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

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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	

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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	

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

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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: _____.

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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: _____

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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"

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APPENDIX 6 :
RESULT OF AAS

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	Action	Sample ID	Q	True Value (mg/L)	Conc. (mg/L)	Abs.	%RSD	SD
1	AUTOZERO	10						
18	STD-1	STD 2		0.4000		0.0778		
19	STD-2	STD 2		0.4000		0.0773		
20	STD-3	STD 2		0.4000		0.0774		
21	**STD-AV**	**STD 2**		**0.4000**		**0.0775**	**0.3414**	**0.000265**
22	STD-1	STD 1		0.2000		0.0304		
23	STD-2	STD 1		0.2000		0.0308		
24	STD-3	STD 1		0.2000		0.0297		
25	**STD-AV**	**STD 1**		**0.2000**		**0.0303**	**1.8375**	**0.000557**
26	STD-1	STD 3		0.8000		0.1489		
27	STD-2	STD 3		0.8000		0.1553		
28	STD-3	STD 3		0.8000		0.1594		
29	**STD-AV**	**STD 3**		**0.8000**		**0.1539**	**3.4269**	**0.005273**
30	BLK-1	Sample Blank				-0.0005		
31	BLK-2	Sample Blank				0.0002		
32	BLK-3	Sample Blank				-0.0002		
33	**BLK-AV**	**Sample Blank**				**-0.0002**	**210.7131**	**0.000351**
34	UNK1-1	007			0.0152	0.0029		
35	UNK1-2	007			0.0089	0.0017		
36	UNK1-3	007			0.0058	0.0011		
37	**UNK1-AV**	**007**			**0.0100**	**0.0019**	**48.2376**	**0.000917**
38	UNK2-1	008			0.0136	0.0026		
39	UNK2-2	008			0.0157	0.0030		
40	UNK2-3	008			0.0152	0.0029		
41	**UNK2-AV**	**008**			**0.0147**	**0.0028**	**7.3471**	**0.000208**
42	UNK3-1	009			0.0121	0.0023		
43	UNK3-2	009			0.0152	0.0029		
44	UNK3-3	009			0.0142	0.0027		
45	**UNK3-AV**	**009**			**0.0136**	**0.0026**	**11.6015**	**0.000306**
46	UNK4-1	010			0.0100	0.0019		
47	UNK4-2	010			0.0047	0.0009		
48	UNK4-3	010			0.0136	0.0026		
49	**UNK4-AV**	**010**			**0.0094**	**0.0018**	**47.4667**	**0.000854**
50	UNK5-1	011			0.0079	0.0015		
51	UNK5-2	011			0.0110	0.0021		
52	UNK5-3	011			0.0089	0.0017		
53	**UNK5-AV**	**011**			**0.0094**	**0.0018**	**17.2927**	**0.000306**
54	UNK6-1	012			0.0100	0.0019		
55	UNK6-2	012			0.0084	0.0016		
56	UNK6-3	012			0.0115	0.0022		
57	**UNK6-AV**	**012**			**0.0100**	**0.0019**	**15.7895**	**0.000300**

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Abs=0.191Conc+ 0 r=0.9985

CONC	ABS
0.4000	0.0775
0.2000	0.0303
0.8000	0.1539

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