



UNIVERSITI PUTRA MALAYSIA

***OCCURRENCE OF LOW MOLECULAR WEIGHT POLYCYCLIC
AROMATIC HYDROCARBONS (PAHs) IN BLOOD SERUMS OF
ADULTS LIVING IN DIFFERENT TRAFFIC VOLUME AREAS IN
KLANG VALLEY***

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FPSK4 2023 26**

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LIVING IN DIFFERENT TRAFFIC VOLUME AREAS IN KLANG VALLEY**



BY

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**Thesis submitted in fulfilment of the requirement for the degree of Bachelor of
Science in Environmental and Occupational Health with Honours from the Faculty
of Medicine and Health Sciences, Universiti Putra Malaysia**

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ABSTRACT

OCCURRENCE OF LOW MOLECULAR WEIGHT POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) IN BLOOD SERUMS OF ADULTS LIVING IN DIFFERENT TRAFFIC VOLUME AREAS IN KLANG VALLEY

SITI MARYAM BINTI CHE RUSLAY

Introduction: This study investigates the occurrence of low molecular weight polycyclic aromatic hydrocarbons (LMW-PAHs) among adults in low and high traffic volume areas. Presently, there is a lack of research in Malaysia that focus on specific molecular weight compound of PAHs in human blood. **Objectives:** This study aimed to compare the concentration of low molecular weight polycyclic aromatic hydrocarbons (LMW-PAHs) in blood serums of adult population living in high traffic volume area and low traffic volume area in Klang Valley, Malaysia and to determine its contributing factors of exposure. **Methodology:** Data on sociodemographic and exposure to PAHs were collected using questionnaire. A total of 203 respondents were involved in this study; 101 exposed group and 102 comparative group. Blood serum samples were extracted using ultrasonication, cleaned-up with solid phase extraction (SPE), quantified by gas chromatography mass spectrometry (GC-MS) to obtain the reading of the concentration of LMW-PAHs compounds. **Results and Discussion:** The findings from Mann-Whitney U test showed that the concentrations of LMW-PAHs in blood serums were significant different between the exposed group and comparative group ($p < 0.05$). The median concentrations of LMW-PAHs in blood serums of exposed group were significantly higher than comparative group. The median concentrations of naphthalene, acenaphthene, acenaphthylene, fluorene, phenanthrene and anthracene in exposed group were 11.16 ng/mL, 2.56 ng/mL, 13.44 ng/mL, 8.54 ng/mL, 3.85 ng/mL and 4.52 ng/mL respectively. While the median concentrations of naphthalene, acenaphthene, acenaphthylene, fluorene, phenanthrene and anthracene in comparative group were 4.99 ng/mL, 9.45 ng/mL, 4.78 ng/mL, 1.43 ng/mL and less than detection limit respectively. The concentrations of naphthalene, fluorene, and anthracene were significant different between respondents with exposure to chemical in working environment and respondents without exposure to chemical in working environment ($p < 0.05$). There were significant differences ($p < 0.05$) between the concentrations of LMW-PAHs in blood serums and the home environment factors; (i) with and without traffic congestion, (ii) with and without open burning activities, (iii) with and without highway near residential area. Meanwhile, there were significant differences ($p < 0.05$) between the concentration of phenanthrene and the status of passive smoker. There were significant differences ($p < 0.05$) between (i) the

concentrations of all target compounds in blood serum and the mode of transportation (ii) the concentrations of acenaphthene, fluorene, and anthracene and diet (eating grilled food) (iii) the concentrations of fluorene, phenanthrene, and anthracene with indoor and outdoor hobbies. **Conclusion:** The concentration of LMW-PAHs in blood serums of exposed group was significantly higher than comparative group. This study suggested the concentrations of LMW-PAHs in blood serums of respondents could be associated with factors such as exposure to traffic congestion, existence of highway near residential area transportation mode and open burning activities.

Keywords: Polycyclic aromatic hydrocarbons (PAHs), blood serums, GC-MS, LMW-PAHs, extraction



ABSTRAK

KEJADIAN BERAT MOLEKUL RENDAH POLISIKLİK AROMATIK HIDROKARBONS (PAHs) DALAM SERUM DARAH GOLONGAN DEWASA YANG TINGGAL DI KAWASAN ISIPADU LALULINTAS YANG BERBEZA DI LEMBAH KLANG

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Pengenalan: Kajian ini menyiasat kejadian berat molekul rendah polisiklik aromatik hidrokarbons (LMW-PAHs) dalam golongan dewasa di kawasan isipadu lalulintas rendah dan tinggi. Pada masa ini, terdapat kekurangan penyelidikan di Malaysia yang memfokuskan kepada sebatian sasaran berat molekul tertentu PAHs dalam darah manusia. **Objektif:** Kajian ini bertujuan untuk membandingkan kepekatan berat molekul rendah polisiklik aromatik hidrokarbon (LMW-PAH) dalam serum darah populasi dewasa yang tinggal di kawasan isipadu trafik tinggi dan kawasan isipadu trafik rendah di Lembah Klang, Malaysia dan untuk menentukan faktor penyumbang pendedahannya. **Metodologi:** Data mengenai sosiodemografi dan pendedahan kepada PAH dikumpul menggunakan soal selidik. Seramai 203 orang responden telah terlibat dalam kajian ini; 101 kumpulan terdedah dan 102 kumpulan perbandingan. Sampel serum darah diekstrak menggunakan ultrasonik, dibersihkan dengan pengekstrakan fasa pepejal (SPE), dikira dengan spektrometri jisim kromatografi gas (GC-MS) untuk mendapatkan bacaan kepekatan sebatian LMW-PAHs. **Keputusan dan Perbincangan:** Hasil kajian daripada ujian Mann-Whitney U menunjukkan bahawa kepekatan LMW-PAH dalam serum darah adalah berbeza secara signifikan ($p < 0.05$) antara kumpulan terdedah dan kumpulan perbandingan. Kepekatan median LMW-PAH dalam serum darah kumpulan terdedah adalah jauh lebih tinggi daripada kumpulan perbandingan. Kepekatan median naftalena, acenaphthene, acenaphthylene, fluorena, fenantrina dan antrasena dalam kumpulan terdedah ialah 11.16 ng/mL, 2.56 ng/mL, 13.44 ng/mL, 8.54 ng/mL, 3.85 ng/mL dan 3.85 ng/mL. Manakala kepekatan median naftalena, acenaphthene, acenaphthylene, fluorene, fenantrina dan antracene dalam kumpulan perbandingan ialah 4.99 ng/mL, 9.45 ng/mL, 4.78 ng/mL, 1.43 ng/mL dan kurang daripada had pengesanan. Kepekatan naftalena, fluorena, dan antrasena adalah berbeza secara signifikan antara responden yang terdedah kepada bahan kimia di persekitaran kerja dan responden tanpa pendedahan kepada bahan kimia di persekitaran kerja ($p < 0.05$). Terdapat perbezaan yang ketara ($p < 0.05$) antara kepekatan LMW-PAH dalam serum darah dan faktor persekitaran rumah; (i) dengan dan tanpa kesesakan lalu lintas, (ii) dengan dan tanpa aktiviti pembakaran terbuka, (iii) dengan dan tanpa lebuhraya berhampiran kawasan perumahan. Manakala, terdapat perbezaan yang signifikan ($p < 0.05$) antara kepekatan fenantrina dan status perokok pasif. Terdapat perbezaan yang ketara

($p < 0.05$) antara (i) kepekatan semua sebatian sasaran dalam serum darah dan cara pengangkutan (ii) kepekatan acenaphthene, fluorene, dan anthracene dan diet (makan makanan panggang) (iii) kepekatan fluorena, fenantrina, dan antrasena dengan hobi dalaman dan luaran. **Kesimpulan:** Kepekatan LMW-PAH dalam serum darah kumpulan terdedah adalah jauh lebih tinggi daripada kumpulan perbandingan. Kajian ini mencadangkan kepekatan LMW-PAH dalam serum darah responden boleh dikaitkan dengan faktor-faktor seperti pendedahan kepada kesesakan lalu lintas, aktiviti pembakaran terbuka, kewujudan lebuh raya berhampiran kawasan perumahan dan mod pengangkutan.

Kata kunci: Polisiklik aromatik hidrokarbons (PAHs), serum darah, GC-MS, LMW-PAH, pengekstrakan



TABLE OF CONTENTS

	Page
DECLARATION	ii
SIGNATURE OF SUPERVISOR/ INTERNAL EXAMINER	iii
ACKNOWLEDGEMENT	iv
ABSTRACT	v
ABSTRAK	vii
CONTENTS	ix
LIST OF TABLES	xii
LIST OF FIGURES	xiii
LIST OF ABBREVIATIONS	xiv
 CHAPTER 1: INTRODUCTION	
1.1 Research Background	1
1.2 Problem Statement	5
1.3 Research Justification	8
1.4 Objectives	10
1.5 Hypothesis	11
1.6 Definition of Variables	11
1.7 Conceptual Framework	14
 CHAPTER 2: LITERATURE REVIEW	
2.1 Polycyclic Aromatic Hydrocarbons	15
2.2 Structures of Polycyclic Aromatic Hydrocarbons	16
2.3 Sources of Polycyclic Aromatic Hydrocarbons	17
2.4 Traffic emissions as sources of PAHs	18
2.5 Route of Exposure	19
2.6 Health Effect from the Exposure	20

CHAPTER 3: METHDOLOGY

3.1	Study Design	21
3.2	Study Location	21
3.3	Study Population	24
3.4	Sampling Technique	26
	3.4.1 Questionnaire	26
	3.4.2 Blood Sampling	26
3.5	Sample Extraction and Analysis	27
	3.5.1 Materials and Standards	27
	3.5.2 Extraction of PAHs in Blood Serums	27
	3.5.3 Lipid Profile Analysis	28
3.6	Analysis of PAHs using Gas Chromatography Mass Spectrometry (GC-MS)	29
3.7	Quality Control	29
	3.7.1 Glassware Cleaning	29
	3.7.2 Calibration of GC-MS	30
	3.7.3 Preparation of Blank	30
	3.7.4 Method Performance	30
3.8	Statistical Analysis	32
3.9	Ethical Consideration	33

CHAPTER 4: RESULTS

4.1	Socio-demographic Information	34
4.2	Contributing Factors of PAHs Exposures	35
	4.2.1 Home Environment Factors	35
	4.2.2 Work Environment Factors	36
	4.2.3 Lifestyles Factors	36
4.3	Blood Lipid Content	38
4.4	Concentration of LMW-PAHs	39
4.5	Association Between Contributing Factors and Concentration of LMW-PAHs	41
	4.5.1 Work Environment Factors	41
	4.5.2 Home Environment Factors	41
	4.5.3 Lifestyles Factors	42

CHAPTER 5: DISCUSSIONS

5.1	Concentration of LMW-PAHs	46
5.2	Association Between Contributing Factors and Concentration of LMW-PAHs	46
5.2.1	Work Environment Factors	46
5.2.2	Home Environment Factors	47
5.2.3	Lifestyles Factors	48

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS FOR FUTURE RESEARCH

6.1	Conclusion	49
6.2	Recommendation	50
6.3	Limitations	50

REFERENCES	51
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APPENDICES	56
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LIST OF TABLES

	Page
Table 1.1 List of the characteristics of LMW-PAHs	4
Table 3.1 Longitude and latitude of study location	20
Table 3.2 Road Traffic Volume Malaysia (RTVM) from 2018-2021	21
Table 3.3 Linear range, correlation coefficient (R ²), limits of detection (LOD), and recoveries and relative standard deviation (RSD).	30
Table 4.1 Socio-demographic information of the respondents (N=203)	32
Table 4.2 Contributing factors among the exposed and comparative group	35
Table 4.3 Lipid Content Information	36
Table 4.4 Concentration of LMW-PAHs detected in blood serum samples of exposed group and comparative group by using Mann-Whitney U Test	38
Table 4.5.1 Median (IQR) of association between the work environment factors and concentration of LMW-PAHs detected in blood serum (ng/mL) by using Mann-Whitney U Test.	41
Table 4.5.2 Median (IQR) of association between the home environment factors and concentration of LMW-PAHs detected in blood serum (ng/mL) by using Mann-Whitney U Test.	42
Table 4.5.3 Median (IQR) of association between the lifestyles factor and concentration of LMW-PAHs detected in blood serum (ng/mL) by using Mann-Whitney U Test and Kruskal-Wallis Test	43

LIST OF FIGURES

	Page
Figure 1.1 Conceptual framework	13
Figure 2.1 Structures of 16 PAHs from three different categories	15
Figure 3.2 Map of Study Location	20



LIST OF ABBREVIATIONS

GS-MS	Gas Chromatography-Mass Spectrometry
LMW-PAHs	Low Molecular Weight Polycyclic Aromatic Hydrocarbons
PAHs	Polycyclic Aromatic Hydrocarbons
US EPA	United State Environmental Protection Agency
SPE	Solid-phase extraction
HPLC	High performance or pressure liquid chromatography
DCM	Dichloromethane

CHAPTER 1

INTRODUCTION

1.1 Research Background

Polycyclic Aromatic Hydrocarbons (PAHs) are a class of organic compound that consists of carbon and hydrogen atoms (Abdel-Shafy & Mansour, 2016). PAHs exist in the environment in a form of aromatic rings structures. This aromatic rings structures of PAHs can be categorized into three groups according to the number of rings. Firstly, low molecular weight PAHs (LMW-PAHs) that consist two to three rings. Next, PAHs that have 4 rings known as medium molecular weight PAHs (MMW-PAHs). Lastly, high molecular weight PAHs (HMW-PAHs) for PAHs that have five to six rings structures (Patel, 2020).

PAHs can be naturally occurring from incomplete combustion of forest fires and volcanic eruptions (Remizovschi et al., 2020) or from the anthropogenic activities that occur such as open burning and automobile exhaust (The Analytical Scientist, 2020). There are several common characteristics of PAHs mainly known as highly lipophilic as they are very soluble in organic solvents compare to water (Singh et al., 2019). PAHs also have a high melting and boiling point and very low aqueous solubility and vapor pressure that result them to be persistence in the environment (Abdel-Shafy & Mansour, 2016).

Based on previous study, sources of PAHs can also be divided into two main categories either pyrogenic PAHs and petrogenic PAHs. These two categories were divided based on their process of PAHs formation depend on a few factors such as temperature, duration and pressure involved during the formation (Abdel-Shafy & Mansour, 2016). High molecular weight (HMW) molecules with 4–7 fused aromatic benzene rings make up pyrogenic PAHs, whereas low molecular weight (LMW) molecules with 2-3 fused aromatic benzene rings make up the majority of petrogenic PAHs (Chiu et al., 2018).

The route of exposure of PAHs can be through three major pathway which are inhalation, ingestion and dermal contact. This exposure might occur through a single route of exposure or occur simultaneously (Abdel-Shafy & Mansour, 2016). Inhalation exposure can be from the breathing of air that contaminated PAHs that produced from incomplete combustion from the environment such as vehicle exhaust emission (Zhang et al., 2021). Both inhalation and dermal exposures mainly based on exposure through the air that already contaminated with the PAHs. Meanwhile, PAHs can be exposed through ingestion through their eating habit such as consuming grilled food (Alomirah et al., 2011).

Health effects of PAHs exposure can be acute (short-term) and chronic (long-term) based on their dose or concentration of exposure, level of persistence in human body and routes of PAHs exposure to human (Abdel-Shafy & Mansour, 2016). Eye discomfort, nausea, diarrhoea, skin irritation, and inflammation are some of the examples of acute health effect that may occur based on high concentration in short duration of time (Abdel-Shafy & Mansour, 2016). Next, the chronic health effects majority will give result to cancer as most of the PAHs compounds have the toxicity effect which are carcinogenicity (Bendtsen et al., 2021). The most cancer that had been reported from the exposure of PAHs are lung cancer and liver cancer (Centre for Environment & Health (BON), 2021).

Previous study mostly focused on general PAHs and there were limited studies that mainly focus on the specific target compounds (Wang et al., 2017). To date, no study has reported the occurrence of PAHs in blood serums of adult population Malaysia as most of the studies were focused on urine samples and children population (Sochacka-Tatara et al., 2018). Thus, this study aimed to compare the LMW-PAHs in blood serums concentration among adult population that lived in low and high traffic volume areas in Klang Valley and to determine the major contributing factors of PAHs exposure. The information of target compounds is summarised in Table 1.1.

Table 1.1: List of the characteristics of LMW-PAHs

Name	Structure and Formula	Molecular Weight (g/mole)	Solubility in water mg/L	Melting point (°C)	Boiling point (°C)	Vapor pressure (mmHg)	Log Kow	Log Koc	Toxicity as per IARC
Naphthalene	 C ₁₀ H ₈	128.17	31	80-26	218	0.087	3.29	2.97	2B
Acenaphthene	 C ₁₂ H ₁₀	154.21	3.8	95	96	4.47 × 10 ⁻³	3.98	3.66	3
Acenaphthylene	 C ₁₂ H ₈	152.20	16.1	92-93	265-275	0.029	4.07	1.40	3
Fluorene	 C ₁₃ H ₁₀	166.22	1.9	116-117	295	3.2 × 10 ⁻⁴	4.18	3.86	3
Phenanthrene	 C ₁₄ H ₁₀	178.23	1.1	100	340	6.8 × 10 ⁻⁴	4.45	4.15	3
Anthracene	 C ₁₄ H ₁₀	178.23	0.045	218	340-342	1.75 × 10 ⁻⁶	4.45	4.15	3

1.2 Problem statement

Centre for Research on Energy and Clean Air (2022) reported Malaysia experiences 32,000 preventable deaths annually as a result of ambient air pollution. PAHs is one of the main pollutants that released from air pollution into the atmosphere because of these environmentally persistent pollutants are mostly introduced in two different fractions: gas phase and bound to particulate matter (Nursanto et al., 2022). Polycyclic Aromatic Hydrocarbons (PAHs) are one of the most important types of environmental pollutants (Chiu et al., 2018).

The primary anthropogenic sources of emission are the burning of agricultural waste and the incomplete combustion or pyrolysis of organic material (i.e., emissions from automobiles, home heating, or cooking); PAHs in air pollution are primarily found bound to particulate matter; when PAHs are present in the gas phase (Centre for Environment & Health (BON), 2021). In Malaysia, the most important source of PAHs in cities expected from the vehicular emissions as reported in the Environmental Quality Report on 2016. Private cars, motorcycles, light, and heavy vehicles are major contributors to bad air quality, especially in the cities (Mohd Shafie & Mahmud, 2020).

The environment is becoming more contaminated with PAHs as a result of increased urbanisation, which raises the risk to human health (Wu et al., 2019). Next, urbanisation and a rise in the number of households considerably increase the emissions of PAHs, which then move to different environmental media like rivers, the sea, and the atmosphere (X. Li et al., 2022). In addition, researchers who examined PAHs concentrations in the soils of two Florida cities discovered that they were particularly high in the central districts and near roads with high traffic volumes (Zhang et al., 2022). The total number of new vehicle registration in Malaysia increased by 51.81% in 2021 (Ministry of Transportation, 2021).

The route of exposure of PAHs can be through three major pathway which are inhalation, ingestion and dermal contact. This exposure might occur through a single route of exposure or occur simultaneously (Abdel-Shafy & Mansour, 2016). Next, the most significant health effect to be expected from inhalation exposure to PAHs is an excess risk of lung cancer (Abdel-Shafy & Mansour, 2016). Yin et al. (2017) also stated that PAH exposure may increase the risk of lung cancer as well as cardiovascular disease (CVD), including atherosclerosis, thrombosis, hypertension, and myocardial infarction (MI).

According to previous study by Jamhari et al. (2014) the number of cars used for daily activities is correlated with the density of industry, residential, and commercial centres in the Klang Valley, according to information on the traffic composition of the Klang Valley released by the Road Transport Department. Lammel et al. (2020) found that it's even probable for ultrafine particles to pass through the membrane and deliver contaminants without dissolving in the lung fluid. In laboratory tests, animals exposed to particular PAHs at high concentrations for extended periods of time experience lung cancer from inhalation, stomach cancer from eating PAHs in food, and skin cancer through skin contact (Rengarajan et al., 2015). Most of the previous studies found that the high amount of traffic pollutants especially PAHs that produce from urban areas (high traffic volume areas) could bound to particulate matter will enter and deposited in the human blood. Hence, in a longer period of time it can give adverse health effect to human.

1.3 Research justification

Malaysia is one of the countries with rapid development on urbanisation. Rapid development including the enhancement of technologies and industries in order to be as the same level with other developed countries. The rapid development is a proactive measure yet this movement also can give negative impact for the short and long-term especially on the environment and human health. Urbanization can result on increase population density at the same time, the number of vehicles also increase (Adriazola-Steil, 2013).

According to the recent research by US EPA (2022) an increasing number of vehicles, can lead to the more pollutant released from the vehicular emissions. PAHs is one of the pollutants that have the characteristic to bound with particulate matter as this pollutant are very ubiquitous in the environment and can give adverse health impact to human (Abdel-Shafy & Mansour, 2016) Besides that, PAHs also can be transmitted not only from vehicle emission as PAHs produced from incomplete combustion. Thus, other sources such as exposed to smoke from smoker, open burning activities, and consume grilled or fried food might give result on deposition of PAHs in human body (Patel et al., 2020; Hamidi et al., 2016).

PAHs presence in the environment in three categories depending on their structures. Low molecular weight of PAH (LMW-PAHs) is one of them that consist of 2 to 3 rings structure (Abdel-Shafy & Mansour, 2016). Based on previous study, most of them were focused on the general PAHs rather than specific target compounds of PAHs. Previous study mostly focused on general PAHs and there were limited studies that mainly focus on the specific target compounds (Wang et al., 2017). To date, there were also no study has reported the occurrence of PAHs in blood serums of adult population in Malaysia as most of the studies were focused on urine samples and children population (Sochacka-Tatara et al., 2018). The concentration of PAHs in the blood is important to understand as the absorption of externally exposed PAHs into the bloodstream and circulating them throughout the body. Biological monitoring of exposure to PAHs is of primary interest, due to the widespread diffusion of these compounds and to their toxicological relevance (Abdel-Shafy & Mansour, 2016). Hence, this study aimed to compare the LMW-PAHs in blood serums concentration among adult population that lived in low and high traffic volume areas in Klang Valley and to determine the major contributing factors of PAHs exposure.

1.4 Objectives

1.4.1 General objective

To compare the concentration of low molecular weight of polycyclic aromatic hydrocarbons (LMW-PAHs) in blood serums of adult population living in high traffic volume area and low traffic volume area in Klang Valley.

1.4.2 Specific objective

1. To determine the sociodemographic background and the contributing factors for the exposure of LMW-PAHs among the adults living in the high and low traffic volume areas in Klang Valley.
2. To compare the concentration of LMW-PAHs in blood serums of the adult population living in the high and low traffic volume areas in Klang Valley.
3. To determine the association between blood serums concentration of LMW-PAHs and contributing factors (work environment factors, home environment factors, lifestyles factors) in adult population living in high and low traffic volume areas in Klang Valley.

1.5 Hypothesis

1. The serums concentration of LMW-PAHs is significantly higher among the adults living in the high traffic volume area compared to low traffic volume area in Klang Valley.
2. There are association between blood serums concentration of LMW-PAHs and contributing factors (work environmental factors, home environment factors, lifestyles factors) in adult population living in the high and low traffic volume areas in Klang Valley.

1.6 Definition of variables

1. Polycyclic Aromatic Hydrocarbons (PAHs) _____

Conceptual definition:

Organic compounds that composed of carbon and hydrogen aromatic rings that are colourless, white, and pale-yellow solid (Abdel-Shafy & Mansour, 2016).

Operational definition:

PAHs concentration is determined from the blood samples collected from the respondents and result of extraction analysis by using gas chromatography mass spectrometry (GC-MS).

2. Blood serum

Conceptual definition:

A clear, pale-yellow liquid extract from the blood separate from blood cells and clotting factors (Biology Online, 2021).

Operational definition:

Blood is collected from the respondents are then placed in the plain tube. Blood serum get from the process of centrifugation and extraction in the laboratory.

3. Traffic Volume

Conceptual definition:

The number of vehicles that pass at a particular location on a road segment or in a lane over a specific time period (Musa et al., 2022).

Operational definition:

In this research, traffic volume will be divided into two categories which are low and high traffic volume areas. The categorisation is based on the station listed in Road Traffic Volume Malaysia 2018 book.



1.7 Conceptual framework

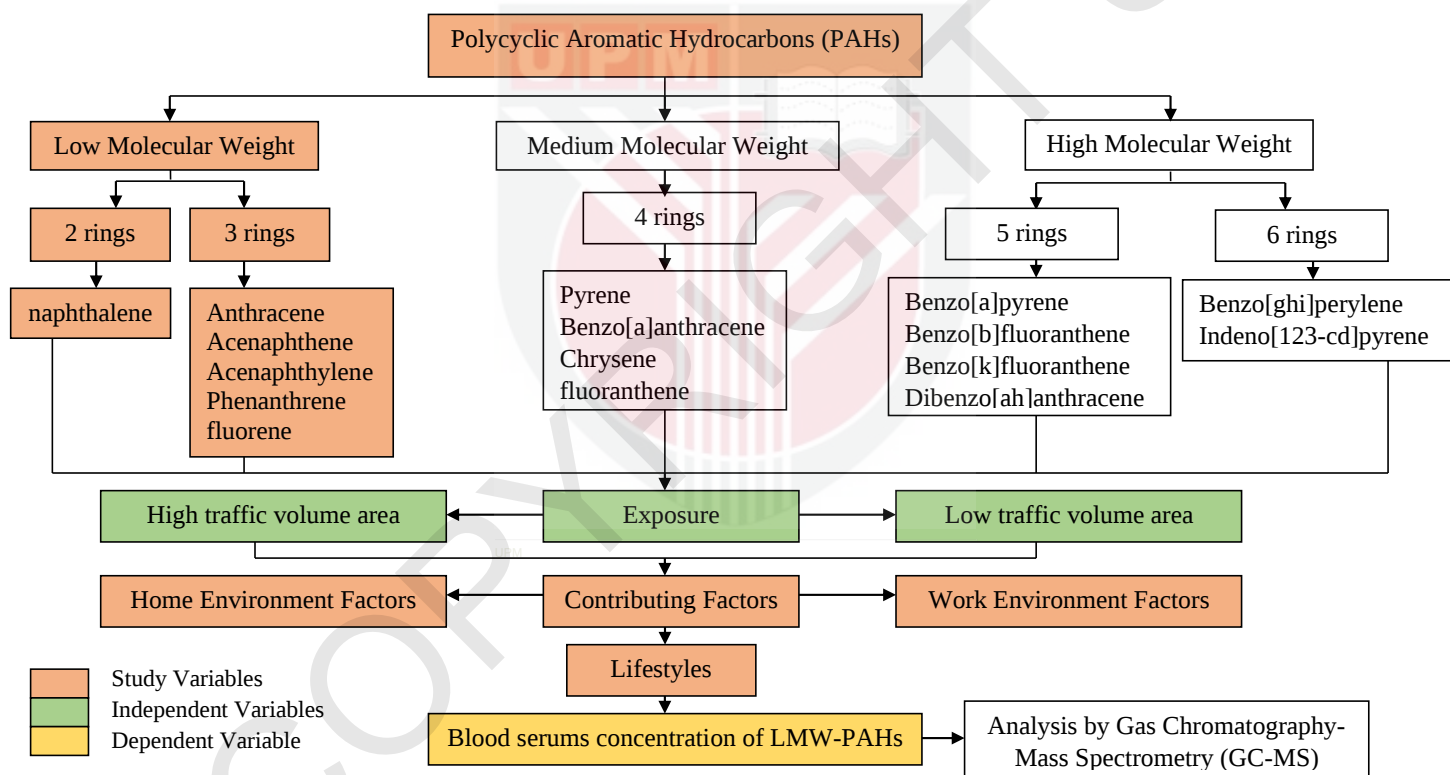


Figure 1.1: Conceptual framework

CHAPTER 2

LITERATURE REVIEW

2.1 Polycyclic aromatic hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) are colourless, white, or pale yellow solid organic substances; they are a very widespread category of several hundred chemically related chemicals with different toxicity and environmental persistence. (Abdel-Shafy & Mansour, 2016). There are several common characteristics of PAHs mainly known as highly lipophilic as they are very soluble in organic solvents compare to water (Y. H. Kim et al., 2016; Singh et al., 2019). PAHs also have a high melting and boiling point and very low aqueous solubility and vapor pressure that result them to be persistence in the environment (Abdel-Shafy & Mansour, 2016). The molecular weight of PAHs has a significant impact on how soluble they are in water (Abdel-Shafy & Mansour, 2016).

2.2 Structures of polycyclic aromatic hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) are a large class of organic compounds containing two or more fused aromatic (benzene) rings (Centre for Environment & Health (BON), 2021). In particular, the term hydrocarbons refer to compounds containing only carbon and hydrogen atoms (Carey, 1998). PAHs exist in the environment in a form of aromatic rings structures. This aromatic rings structures of PAHs can be categorized into three groups according to the number of rings. Firstly, low molecular weight PAHs (LMW-PAHs) that consist two to three rings. Next, PAHs that have 4 rings known as medium molecular weight PAHs (MMW-PAHs). Lastly, high molecular weight PAHs (HMW-PAHs) for PAHs that have five to six rings structures (Patel, 2020). For example, there is one type of PAHs that have two rings named naphthalene, that will be categorized under LMW-PAHs (Figure 2.1).

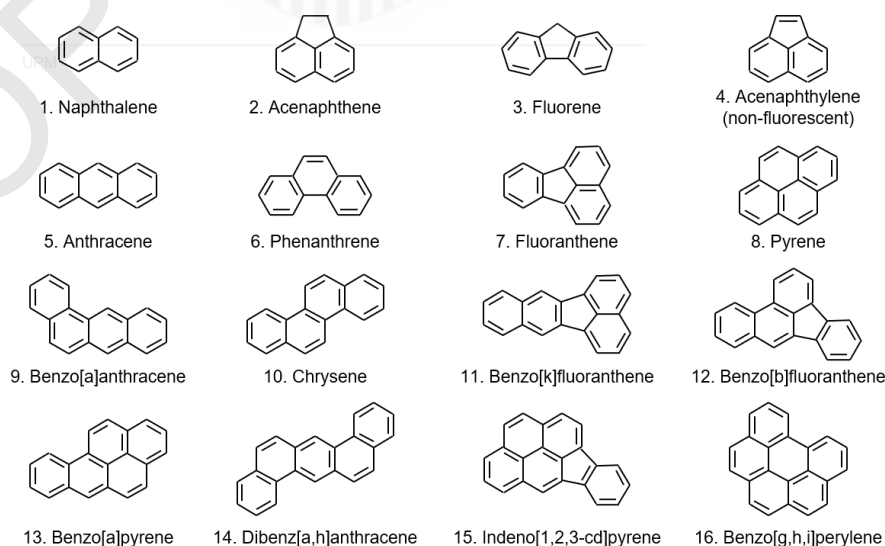


Figure 2.1: Structures of 16 PAHs from three different categories

2.3 Sources of polycyclic aromatic hydrocarbons (PAHs)

PAHs can be naturally occurring from incomplete combustion of forest fires and volcanic eruptions (Remizovschi et al., 2020) or from the anthropogenic activities that occur such as open burning and automobile exhaust (The Analytical Scientist, 2020). The primary causes of PAH pollution are anthropogenic emission sources, which can be categorized into four categories: industrial, mobile, household, and agricultural emission sources (Patel et al., 2020). Specifically, identified PAHs are traffic tracers suggesting that vehicular emission was one of its main sources (Nursanto et al., 2022). In addition, PAHs are also can be formed through domestic and industrial food processing like roasting, toasting, drying, grilling, frying, baking, and barbecuing (Patel et al., 2020).

Based on previous study, sources of PAHs can also be divided into two main categories either pyrogenic PAHs and petrogenic PAHs. These two categories were divided based on their process of PAHs formation depend on a few factors such as temperature, duration and pressure involved during the formation (Abdel-Shafy & Mansour, 2016). High molecular weight (HMW) molecules with 4–7 fused aromatic benzene rings make up pyrogenic PAHs, whereas low molecular weight (LMW) molecules with 2-3 fused aromatic benzene rings make up the majority of petrogenic PAHs (Chiu et al., 2018). The petrogenic PAHs derived from contaminated petroleum products utilized by the submarine, whereas the pyrogenic PAHs were produced as a result of the fire event (Z. Wang et al., 2008). Pyrogenic PAHs are formed whenever

organic substances are exposed to high temperatures under low oxygen or no oxygen conditions, while petrogenic PAHs are formed due to any process that related to lower temperatures such as crude oil maturation (Abdel-Shafy & Mansour, 2016).

2.4 Traffic emissions as sources of PAHs

Most of previous study that had been conducted, stated that traffic emissions as the main contributor to PAHs exposure to the atmosphere. Ma et al. (2010) found that traffic exhaust was a significant source to the atmospheric PAHs, accounting for 59% of all detected PAHs in urban city. PAHs have its characteristics that can easily bound to the pollutants in the atmosphere, especially the particulate matter. The majority of PAHs produced from stationary sources are found to be attached to fine particles, however their sizes are a little bit greater than those of PAHs released from vehicular emission (Yang et al., 2021). Besides, principal component analysis (PCA) suggests that the primary cause of PAH contamination in soil at urban traffic areas in Dhanbad is vehicle emission (Suman et al., 2016). Jamhari et al. (2014) stated that with the exception of samples taken during the northeast monsoon, the concentration values of $\Sigma 16$ PAHs at the PJ station were 42.9% and 28.9% higher than the values for the samples collected in KL and BG, respectively. Due of PJ's function as an industrial, commercial, and residential district, there is a higher traffic density there.

2.5 Route of exposure

Basically, there are four main routes of exposure of pollutants which are inhalation, ingestion, dermal contact and injection. PAHs exposure to human may occur due to inhalation, dermal exposure or the ingestion of food contaminated with PAHs (Rengarajan et al., 2015; Centre for Environment & Health (BON), 2021). The main route of PAHs exposures in the general population is from breathing ambient and indoor air, eating food containing PAHs, smoking cigarettes, or breathing smoke from open fireplaces. This exposure could happen simultaneously or through a single route of exposure (Abdel-Shafy & Mansour, 2016). The majority of these exposures, such as drinking untreated water, eating grilled and smoked food, smoking, are non-occupational settings (Patel et al., 2020). Inhalation exposure can be from the breathing of air that contaminated PAHs that produced from incomplete combustion from the environment such as vehicle exhaust emission (Zhang et al., 2021). Moreover, PAHs can be exposed through ingestion through their eating habit such as consuming grilled food (Alomirah et al., 2011).

2.6 Health effect from the exposure

Health effects of PAHs can be classified into two categories which are acute and chronic health effect (Abdel-Shafy and Mansour, 2016). Acute health effects include eye irritation, vomiting, diarrhoea, confusion, skin irritation, and inflammation, meanwhile, chronic health consequences can cause asthma-like symptoms, eye cataracts, kidney and liver damage, breathing difficulties, weakened immune systems, and lung malfunctions (Abdel-Shafy and Mansour, 2016). Due to PAH exposure, both males and females may have a variety of non-cancer health impacts associated to the reproductive system, including changes in sperm quality, testicular function, and egg viability, as well as DNA damage in oocytes, ovarian damages, and other reproductive illnesses (Patel et al., 2020).

According to epidemiological research, PAHs are linked to poorer lung function, increasing asthma, higher risks of obstructive lung problems, and cardiovascular diseases; significant associations between ambient PAHs and breast cancer, paediatric cancers, and lung cancer have been found. (Centre for Environment & Health (BON), 2021). In addition, the most significant health effect to be expected from inhalation exposure to PAHs is an excess risk of lung cancer (Abdel-Shafy & Mansour, 2016). Numerous PAHs are immunotoxin, mutagenic, carcinogenic, and teratogenic to living organisms, including humans, animals, and microorganism (Patel et al., 2020). Long-term studies have indicated an elevated risk of malignancies, primarily of the skin and lungs but also of the bladder and gastrointestinal system. (S. W. Wang et al., 2019). Overall, the present scientific evidence suggests that the PAHs in ambient air are associated with increased cancer incidence in exposed populations (Centre for Environment & Health (BON), 2021).

CHAPTER 3

METHODOLOGY

3.1 Study design

A comparative cross-sectional study was conducted for this study to compare the concentration of LMW-PAHs in blood serums of adult population that living in high traffic volume area and low traffic volume area in Klang Valley, Selangor, Malaysia.

3.2 Study location

The study is conducted at two selected locations of lowest and highest traffic volume areas in Klang Valley, Selangor, Malaysia. The selected location was according to the Road Traffic Volume Malaysia 2018 report. Station BR807 in Kuala Lumpur was listed as the highest traffic volume station in Malaysia while station BR613 in Hulu Langat was listed as the lowest traffic volume station in Malaysia. The study location for highest traffic volume area was located at the Kondo Rakyat, Kuala Lumpur. While, second study location for lowest traffic volume area was located at Kampung Sungai Lui, Hulu Langat. Each of the study location were selected based on 5 km radius from the road traffic volume station. The study location and information on road traffic volume station were illustrated in Figure 3.1, Table 3.1 and Table 3.2.

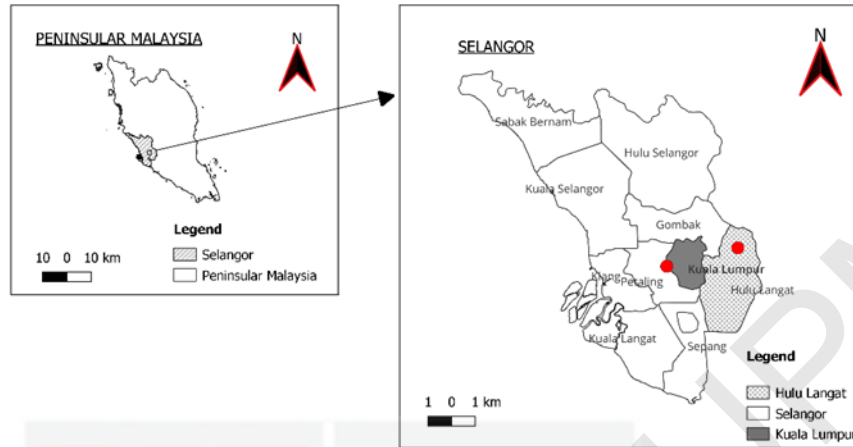


Figure 3.1: Map of study location

Table 3.1: Longitude and latitude of study location

Location	Station Number	Latitude	Longitude
Hulu Langat	BR604	2.9991781	101.7850937
	BR605	2.8721868	101.8705492
	BR608	3.0887500	101.7923056
	BR609	2.9683056	101.7897500
	BR610	2.9534167	101.8460556
	BR611	2.9393990	101.8369410
	BR612	2.9459499	101.8524121
	BR613*	3.1339720	101.9161390
	BR614	2.9733950	101.8311680
Petaling	BR801	3.2066310	101.5620250
	BR802	3.1005030	101.5885100
	BR803	3.0675880	101.5560910
	BR804	3.0851210	101.5545700
	BR806	3.0835180	101.6069030
	BR807**	3.1119700	101.6561250
	BR811	3.0761900	101.5572320
	BR813	3.0302030	101.5749370
	BR814	3.0319380	101.5693160
	BR815	3.0503060	101.6442150

* Lowest traffic volume; ** Highest traffic volume

Table 3.2: Road Traffic Volume Malaysia (RTVM) from 2018-2021

Location	Station Number	16-Hours Traffic Volume			
		2018	2019	2020	2021
Hulu Langat	BR604	N/A	49444	40855	33772
	BR605	10909	11739	10099	3003
	BR608	27978	30097	25886	22821
	BR609	42470	36119	30331	22379
	BR610	9663	12156	11482	10424
	BR611	22144	28254	25948	14636
	BR612	26231	20769	19519	15212
	BR613*	615	N/A	N/A	N/A
	BR614	65548	55427	46871	40765
Petaling	BR801	78439	82525	62836	19578
	BR802	145304	91153	77868	57162
	BR803	131542	64299	55242	32320
	BR804	166810	80273	70018	47423
	BR806	184555	147658	118323	89997
	BR807**	211453	203206	163555	102181
	BR811	17931	23922	20986	21999
	BR813	85368	N/A	N/A	N/A
	BR814	81481	59689	50564	31724
	BR815	74825	101026	81843	60325
	BR816	N/A	60245	58558	46609
	BR817	N/A	40415	35105	33923

* Lowest traffic volume; ** Highest traffic volume

3.3 Study population

The study population was adult that living in Kondo Rakyat, Kuala Lumpur and Kampung Sungai Lui, Hulu Langat. The study population consisted of adult living in Kondo Rakyat, Kuala Lumpur served as exposed group. Meanwhile, adult living in Kampung Sungai Lui, Hulu Langat served as comparative group. The respondents were approached personally, briefed, and asked for consent before participating in this study. The inclusion criteria were set to determine the adult that can participate in this study and only those who fulfil the inclusion criteria were included in the study. Below are the inclusion criteria that have been set for both study location:

- (i) Adult population with age between 18 to 74 years old;
- (ii) Participants that live and work in the same study location.

The exclusion criteria were set to exclude adults with certain criteria from this study. The exclusion criteria are as follow:

- (i) Adults who are had smoking habits.
- (ii) Adults who are their occupational exposure to PAHs. For example, aluminium production, rubber tire manufacturing or cement manufacturing.

The sample size was calculated according to the formula by (Lemeshow et al., 1990):

$$n = \frac{\left\{ Z_{\frac{\alpha}{2}} \sqrt{2\bar{P}(1-\bar{P})} + Z_{1-\beta} \sqrt{P_1(1-P_1) + P_2(1-P_2)} \right\}^2}{(P_1 - P_2)^2} \quad \text{Eq. 3.1}$$

Where,

$$Z_{\frac{\alpha}{2}} = 1.96$$

$$Z_{1-\beta} = 0.842$$

$$\bar{P} = \frac{P_1 + P_2}{2}$$

P_1 = estimated population (comparative group)

P_2 = estimated population (exposed group)

The value for P_1 (0.88) and P_2 (0.99) were based on the study by (Lai et al., 2005) on exposure to traffic exhausts and oxidative DNA damage of exposed group (n = 47) compared to reference group (n = 24). Below is the calculation of the sample size:

$$n = \frac{\left\{ 1.96 \sqrt{2(0.935)(1-0.935)} + 0.842 \sqrt{0.88(1-0.88) + 0.99(1-0.99)} \right\}^2}{(0.88 - 0.99)^2}$$

$$n = 78 (+16)$$

$$n = 94$$

The sample size was increased by 20% to cover for any lost by taking into consideration of missing data and loss of respondents. Hence, the final numbers of respondents are 94 for each group, which make up to 188 respondents for both exposed and comparative group.

3.4 Sampling technique

3.4.1 Questionnaire

Questionnaires were distributed to the respondents to obtain information such as sociodemographic information, exposure of polycyclic aromatic hydrocarbons (PAHs) and dietary status. Informed consent was obtained from each respondent to the community health survey and blood sampling. The validity of the questionnaire was based on a pilot study at Seri Kembangan where the questionnaires were distributed to 20 participants and they were able to understand and answer all questions. The results of the reliability test showed that the Cronbach alpha of the questionnaire was 0.822.

3.4.2 Blood sampling

Ten millilitres of blood samples were collected from each respondent by certified medical personnel from Universiti Putra Malaysia. Serum samples were collected and extracted according to the method of (Yin et al., 2017). The collected blood samples were placed in plain tubes. The collected samples were refrigerated immediately at 4°C after collection and transported to the laboratory. Afterwards, the samples were centrifuged at 3000 rpm for 10 min to obtain the blood serum. The serum layer was transferred into the borosilicate glass vials using the pipette, sealed with parafilm and stored at -20°C until further extraction and analysis.

3.5 Sample Extraction and Analysis

3.5.1 Materials and Standards

Analytical grade solvents (methanol, acetone, dichloromethane (DCM), and n-hexane) were used for glassware cleaning while HPLC grade solvents (DCM, and n-hexane) were used for the process of extraction. Methanol and acetone were purchased from R & M Chemicals (UK), while analytical and HPLC grade DCM and n-hexane were purchased from Merck (Darmstadt, Germany). The PAHs standard mixture (EPA 610 mix) were sourced from Supelco (USA). Chrysene-d12, perylene-d12 and phenanthrene-d10 (Supelco, USA) were applied as representative of internal standards (IS) at the beginning of extraction procedure.

3.5.2 Extraction of PAHs in blood serums

Two millilitre of serum sample was added in a 30 mL glass centrifugation tubes and spiked with 50 μ L internal standards (IS). Then, 0.5 mL of formic acid, 2.5 mL of ethanol and 10 mL of n-hexane/ DCM (1:1, v/v) were then added into the tubes. Then, the mixture was mixed using a vortex mixer and sonicated using ultrasonic bath for 10 min. Next, the mixture was centrifuged at 2000 rpm for 10 min. The organic phase was transferred into a new glass tube by using pipette. The process of extraction was repeated two times. The volume of combined extract was reduced to approximately 1 mL using

rotary evaporator. The silica SPE cartridges (LiChrolut® RP-18, Merck, Germany) were conditioned by 70 mL of n-hexane/DCM (1:1, v/v). The extracts were loaded and passed through the SPE cartridges. Next, the SPE cartridges were eluted using 70 mL of n-hexane/DCM (1:1, v/v) and the eluate were collected into a glass tube. The extract was evaporated to near dryness and transferred to 2 mL GC Vial. The extract was reconstituted with 500 µL n-hexane, sealed with parafilm and sent for gas chromatography mass spectrometry (GC-MS) analysis.

3.5.3 Lipid Profile Analysis

Five hundred microlitre of blood serum from each sample was analysed for its lipid profile. There were 3 µL of blood serum used to determine the total cholesterol (CHOL) and triglycerides (TG) level respectively. The Cholesterol Reagent Kit (Enzyme Method) (Dirui, China) and Triglycerides Reagent Kit (Oxidase Method) (Dirui, China) were used during the procedure of lipid profile analysis. Total serum lipid concentration was calculated based on (Yin et al., 2017):

$$TL(gL^{-1}) = 1.12 \times CHOL(gL^{-1}) + 1.33 \times TG(gL^{-1}) + 1.48 \quad \text{Eq. 3.2}$$

3.6 Analysis of PAHs using GC-MS

All samples were analysed using GC-MS (Agilent Masshunter) in order to obtain the concentration of target LMW-PAHs compounds (naphthalene, acenaphthene, acenaphthylene, fluorine, phenanthrene, and anthracene). One millilitre of pre-concentrated sample was injected into the machine. The concentration of PAHs were quantified using selective ion monitoring (SIM) mode and the process of quantification was performed using standard curve of each target compound by Agilent Masshunter Quantification Software (Agilent Inc. Palo Alto, CA, USA). External calibration was conducted in order to assess the concentrations of individual PAHs. Besides that, 1 mg/L of internal standard mixtures were added at the beginning of the extraction procedure.

3.7 Quality Control

3.7.1 Glassware Cleaning

All glassware were cleaned according to US EPA Method (US EPA, 1999). All glassware were soaked in Decon 90 and rinsed with tap water. Then, the glassware was immediately cleaned with methanol, acetone, DCM, and n-hexane respectively and repeated for three times to ensure the glassware were free from contaminants. Then, the glassware was sealed with aluminium foil to avoid from any dusts or contaminants. After that, all the sealed glassware were dried in oven at 60°C.

3.7.2 Calibration of GC-MS

The calibration curves were used to determine the linearity. The equipment was calibrated with each analyte using four-point calibration curve at the range of 500 to 3000 ng/mL. The peak area ratio of PAHs vs nominal concentrations of the analytes was used to create calibration standards for each concentration.

3.7.3 Preparation of blank

The internal standard was spiked into blank sample (serum sample) and each batch of samples testing was run throughout the whole sample preparation and extraction process to test for any potential background contaminants in the sample. Internal standards (chrysene-d12, phenanthrene-d12, and perylene-d10) were applied to obtain the recovery of the target compound in this study.

3.7.4 Method performance

A spiked blank sample was included and analysed with each batch of samples to monitor the potential contamination of the process. Standards of target compounds were used in order to inspect the extraction and clean-up performance. Recovery rate were calculated using Equation (3.3), (Agus et al., 2020):

$$Recovery (\%) = \left(\frac{C_{spiked} - C_{unspiked}}{C_{added}} \times 100 \right) \quad \text{Eq 3.3}$$

C_{spiked} is the concentration of PAHs quantified in the sample spiked with a mixture of IS, $C_{unspiked}$ is the concentration of PAHs quantified in the blank sample, and C_{added} is the known PAHs concentration that was added to the sample. Concentration of LMW-PAHs in blood serum were calculated using Equation (3.4):

$$C_{blood (ng/ml)} = \left(\frac{C_{ex} \times V_{ex}}{V_s} \right) \quad \text{Eq. 3.4}$$

C_{blood} is the concentration of LMW-PAHs quantified in the blood serum (ng/mL), C_{ex} is the concentration of LMW-PAHs quantified in extract (ng/mL), V_{ex} are the volume of sample extract (0.5 mL), and V_s is the volume of blood serum (2 mL).

The method's sensitivity was determined by looking at the limits of detection (LOD). Five separate measurements of blank samples and their standard deviation were used to define the limit of detection (LOD) for each PAH (Hisamuddin et al., 2022). LOD were derived using the concentration of analyte that produces a signal equivalent to three or two times the standard deviation of the blank sample ($3 \times \sigma_{blank}$ or $2 \times \sigma_{blank}$) (Araujo, 2009; Farhadian et al. 2010). The recovery efficiencies for the internal standards were 57-71% for chrysene-d12, 86-128% for phenanthrene-d12, and 69-101% for perylene-d10. The LOD for individual LMW-PAHs compounds ranged from 1.96 to 2.38 ng/mL. The results for recovery and limits of detection (LOD) are illustrated in the Table 3.3.

Table 3.3: Linear range, correlation coefficient (R²), limits of detection (LOD), and recoveries and relative standard deviation (RSD).

Target Compounds	Linear Range (ng/mL)	R²	LOD (ng/mL)	Recovery ± RSD (%) (n=3)
NAP	500-3000	0.9998	1.96	76.86±7.29
ACE	500-3000	0.9998	1.54	88.82±8.95
ACY	500-3000	0.9996	2.05	95.37±14.34
FLO	500-3000	0.9999	0.74	101.36±10.09
PHE	500-3000	0.9991	0.32	98.03±9.45
ANT	500-3000	0.9991	2.38	103.3±13.11

NAP - Naphthalene, ACE - Acenaphthene, ACY – Acenaphthylene, FLO – Fluorene, PHE – Phenanthrene, ANT – Anthracene

3.8 Statistical Analysis

Descriptive analysis was performed to describe respondents' socio-demographic, concentration of LMW-PAHs in blood serum, and contributing factors (work environment factors, home environment factors, and lifestyles factors). The concentration of LMW-PAHs in blood serum of exposed and comparative group were compared using the Mann–Whitney U test. Next, the significant differences between the concentration of LMW-PAHs in serums and contributing factors were determined using the Mann-Whitney U test and Kruskal–Wallis test. Statistical data were performed using Statistical Package for the Social Sciences (SPSS) version 27 (IBM Corp, New York, USA).

3.9 Ethical Consideration

Ethical approval for this study was granted by the Ethics Committee for Research Involving Human Subjects Universiti Putra Malaysia (JKEUPM-2018-285). The respondents were explained on the purpose of this study and how the study will carry out. Written consent forms from the respondents were obtained before the study to make sure that the respondents are willing to participate in this study. Short briefing to the study subjects was carried out to ensure they understand the procedure and instruction before the data collection process. All the collected data from this study were confidential.

CHAPTER 4

RESULTS

4.1 Socio-demographic information

The respondents' socio-demographics information was tabulated in table 4.1. All recruited respondents were lived and worked at the study location. Respondents' age ranged from 18 to 74 years old. For the marital status of exposed group, 20.2% of them were single, 60.6% were married, and 19.2% were widow or divorced. Meanwhile, 18.6% of the comparative respondents were single, 70.6% were married and 10.8% were widow or divorced. Most of the exposed respondents (56.4%) had secondary education, while 8.5% of them had primary school and tertiary education. Meanwhile, 59.8% of comparative group had secondary education and 7.8% of them had tertiary education.

Table 4.1: Socio-demographic information of the respondents (N=203)

Variables	Median (IQR)	
	Exposed Group (<i>n</i> = 101)	Comparative Group (<i>n</i> = 102)
Age (years)	4.00(2.00-5.00)	4.00(.00-5.00)
Frequency, <i>n</i> (%)		
18-27	12 (11.9)	11 (10.8)
28-37	14 (13.9)	11 (10.8)
38-47	15 (14.9)	18 (17.6)
48-57	17 (16.8)	32 (31.4)
58-67	30 (29.7)	23 (22.5)
68-77	13 (12.9)	7 (6.9)

Variables	Categories	Frequency, <i>n</i> (%)	
Groups		Exposed Group (<i>n</i> = 101)	Comparative Group (<i>n</i> =102)
Gender	Male	36 (35.6)	37 (36.3)
	Female	65 (64.4)	65 (63.7)
Race	Malay	98 (99.0)	101 (99.0)
	Indian	1 (1.0)	0 (0.0)
	Chinese	0 (0.0)	1(1.0)
Marital Status	Single	20 (20.2)	19 (18.6)
	Married	60 (60.6)	72 (70.6)
	Widow/Divorced	19 (19.2)	11 (10.8)
Educational Background	No Education	11 (11.7)	11 (10.8)
	Primary School	8 (8.5)	10 (9.8)
	Secondary School	53 (56.4)	61 (59.8)
	Pre-University	14 (14.9)	12 (11.8)
	Tertiary Education	8 (8.5)	8 (7.8)

4.2 Contributing factors of PAHs exposure

4.2.1 Home environment factors

Table 4.2 listed the potential contributing factors for the exposure of PAHs that reported by respondents. There were three major contributing factors namely home environment factors, work environment factors and lifestyles factors. For the home environment factors, there were 77.7% of respondents in exposed group exposed to traffic congestion, while there were no respondents exposed to traffic congestion in comparative group. There were 46.2% of respondents in exposed group exposed to open burning activities in comparison to 98.0% of respondents in comparative group exposed to open burning activities. There were 33.7% of passive smokers in exposed group compared to

48.0% of passive smokers in comparative group. There were 92.7% of respondents in exposed group with highway nearby their residential areas, while all respondents (100%) in comparative group without highway nearby their residential area.

4.2.2 Work environment factors

For the work environment factors, there were 63.3% of respondents in exposed group and 55.8% in comparative group worked indoor. There were 36.7% of respondents in exposed group and 44.2% in comparative group worked outdoor. More than half of the respondents worked less than or equal to eight hours per day in both exposed and comparative group. There were 16.4% of exposed respondents and 4.3% of comparative respondents exposed to chemical in working environment.

4.2.3 Lifestyles factors

There were 60.0% of respondents in exposed group and 77.3% in comparative group had outdoor activities as hobbies. For the mode of transportation among the exposed population, most of them used car (27.6%) compared to other transportations such as motorcycle (21.8%), walking (24.1%), bus (5.3%) and train (1.1%). Meanwhile, most of the respondents in comparative group used motorcycle (46.5%) to commute compared to car (43.6%), walking (7.9%) and bus (2.0%). Most of the respondents had grilled food in their daily diet in both exposed (90.6%) and comparative group (76.0%). Also, most of the respondents in exposed (85.4%) and comparative group (80.0%) had indoor grilling and frying as one of their cooking methods.

Table 4.2: Contributing factors among the exposed and comparative group

Contributing Factors		Exposed Group, <i>n</i> (%)	Comparative Group, <i>n</i> (%)
Home environment factor			
Exposure to traffic congestion			
	Yes	73 (77.7)	0 (0.0)
	No	21 (22.3)	102 (100.0)
Open Burning activities			
	Yes	43 (46.2)	98 (98.0)
	No	50 (49.5)	2 (2.0)
Passive smokers			
	Yes	32 (33.7)	49 (48.0)
	No	63 (66.3)	53 (52.0)
Existence of highway			
	Yes	89 (92.7)	0 (0.0)
	No	7 (7.3)	102 (100.0)
Work environment factor			
Work location			
	Outdoor	22 (36.7)	34 (44.2)
	Indoor	38 (63.3)	43 (55.8)
Work duration			
	≤ 8 hours	43 (75.4)	47 (72.3)
	> 8 hours	14 (24.6)	18 (27.7)
Exposure to chemicals in working Environment			
	Yes	11 (16.4)	4 (4.3)
	No	56 (83.6)	88 (95.7)
Lifestyles			
Hobbies			
	Outdoor	61 (81.3)	90 (92.8)
	Indoor	14 (18.7)	7 (7.2)
Transportation mode			
	Car	24 (27.6)	44 (43.6)
	Motorcycle	19 (21.8)	47 (46.5)
	Train	1 (1.1)	0 (0.0)
	Bus	22 (5.3)	2 (2.0)
	Walking	21 (24.1)	8 (7.9)

Diet (grilled food)	Yes	87 (90.6)	76 (76.0)
	No	9 (9.4)	24 (24.0)
Cooking mode (indoor grilling and frying)	Yes	82 (85.4)	80 (80.0)
	No	14 (14.6)	20 (20.0)

4.3 Blood lipid content

Table 4.3 summarised the result of the lipid content (total cholesterol, triglycerides and total lipids concentration) in the serums of exposed group and comparative group. The median concentrations of total cholesterol, triglycerides and total lipids for exposed group were 2.16 g/L, 1.61 g/L, and 6.04 respectively. The median concentration of total cholesterol, triglycerides and total lipids for comparative group were 2.15 g/L, 0.97 g/L, and 5.28 respectively.

Table 4.3: Lipid Content Information

Lipid Content	Median ± IQR		Minimum		Maximum	
	Exposed Group (n=101)	Comparative Group (n=102)	Exposed Group (n=101)	Comparative Group (n=102)	Exposed Group (n=101)	Comparative Group (n=102)
Total Cholesterol (g/L)	2.16±0.84	2.15±0.50	0.38	1.47	4.70	3.59
Triglycerides (g/L)	1.61±1.16	0.97±1.69	0.43	0.01	5.38	5.31
Total Lipids (g/L)	6.04±2.31	5.28±2.05	2.56	3.37	11.23	12.57

4.4 Concentration of LMW-PAHs

Table 4.4 reported the concentration of LMW-PAHs detected in blood serum of exposed and comparative group. The concentrations of LMW-PAHs in serums were significant different between the exposed group and comparative group. The median concentration of naphthalene, acenaphthene, acenaphthylene, fluorene, phenanthrene and anthracene in blood serums of exposed group were 11.16 ng/mL, 2.56 ng/mL, 13.44 ng/mL, 8.54 ng/mL, 3.85 ng/mL and 4.52 ng/mL respectively. While the median concentration of naphthalene, acenaphthene, acenaphthylene, fluorene, phenanthrene and anthracene in serums of comparative group were 4.99 ng/mL, 9.45 ng/mL, 4.78 ng/mL, 1.43 ng/mL and less than detection limit respectively. Both exposed group and comparative group had the highest median concentration of acenaphthylene which were 13.44 ng/mL and 9.45 ng/mL respectively. Meanwhile, the lowest median concentrations in exposed and comparative group were acenaphthene (2.56 ng/mL) and phenanthrene (1.43 ng/mL) respectively. Anthracene was not detected in the blood serums of comparative group. All LMW-PAHs were significantly higher in the blood serum of exposed group with a p -value < 0.05 .

Table 4.4: Concentration of LMW-PAHs detected in blood serum samples of exposed group and comparative group by using Mann-Whitney U Test

Target Compounds	Median ^a (IQR), (ng/ml)		Frequency of Detection, <i>n</i> (%)		<i>p</i> -Value	Z-Score
	Exposed Group	Comparative Group	Exposed Group	Comparative Group		
Naphthalene	11.16 (6.09)	4.99 (1.15)	100 (99.0)	100 (98.0)	<0.001*	-9.970
Acenaphthene	2.56 (2.20)	N/A (2.14)	79 (78.2)	46 (45.1)	<0.001*	-7.214
Acenaphthylene	13.44 (8.95)	9.45 (1.69)	101 (100.0)	102 (100.0)	<0.001*	-6.378
Fluorene	8.54 (4.84)	4.78 (1.18)	101 (100.0)	102 (100.0)	<0.001*	-9.280
Phenanthrene	3.85 (0.42)	1.43 (0.64)	101 (100.0)	102 (100.0)	<0.001*	-12.309
Anthracene	4.52 (0.58)	ND	101 (100.0)	ND	<0.001*	-13.172

*Sig. difference $p < 0.05$; ND: Not detected; ^a Median was calculated based on the frequency of detection (*n*), instead of total number of samples expose group (*n* = 101) and comparative group (*n* = 102)

4.5 Association between contributing factors and concentration of LMW-PAHs

4.5.1 Work environment factors

Table 4.5.1 summarised the association between the work environment factors and concentration of LMW-PAHs detected in blood serum. Based on the work location, there was no significant difference between the indoor and outdoor working environment and the concentration of all LMW-PAHs (p -value > 0.05). Next, there were also no significant differences of work duration (≤ 8 hours and > 8 hours) and the concentration of all LMW-PAHs ($p > 0.05$). Meanwhile, there were significant differences between (naphthalene, fluorene and anthracene) and exposure to chemical among respondents in working environment.

4.5.2 Home environment factors

Table 4.5.2 reported the association between the home environment factors and the concentration of LMW-PAHs detected in blood serum. There were significant differences between the exposure to traffic congestion, open burning activities, existence of highway nearby the residential areas and the concentration of all LMW-PAHs ($p < 0.05$). Meanwhile, only phenanthrene in blood serum was significantly different between passive and non-passive smokers ($p = 0.025$).

4.5.3 Lifestyles factors

Table 4.5.3 illustrated the association between the lifestyle factors and the concentration of LMW-PAHs detected in blood serum. Based on the lifestyle factor, there was no significant different between the mode of cooking (with and without indoor grilling and frying) and the concentration of all LMW-PAHs ($p>0.05$). Next, there was significant different between the diet (with and without consumption of grilled food) and the concentration of (acenaphthene, fluorene and anthracene). There were also significant differences between hobbies and three of the target compounds (fluorene, phenanthrene, and anthracene). There were significant differences between the transportation mode (commute with car, motor, train, bus and walk) and the concentration of all LMW-PAHs.

Table 4.5.1: Median (IQR) of association between the work environment factors and concentration of LMW-PAHs detected in blood serum (ng/mL) by using Mann-Whitney U Test

Target compounds	Work location			Work duration			Exposure to chemical in working environment		
	Outdoor (n=56)	Indoor (n=81)	P-Value	≤ 8 hours (n=90)	> 8 hours (n=32)	P-Value	Yes (n=15)	No (n=144)	P-Value
NAP	5.56 (3.44)	6.46 (6.08)	0.197	6.03 (5.23)	6.18 (5.93)	0.942	10.52 (6.46)	5.56 (3.93)	0.003*
ACE	1.82 (2.50)	1.87 (2.80)	0.694	1.89 (2.76)	1.63 (2.48)	0.353	2.50 (3.50)	1.65 (2.46)	0.120
ACY	9.72 (4.38)	10.25 (8.52)	0.669	9.79 (6.18)	10.20 (6.84)	0.606	9.62 (4.88)	10.09 (7.07)	0.544
FLO	5.60 (2.75)	5.43 (4.56)	0.572	5.50 (3.91)	5.45 (4.04)	0.250	7.76 (5.64)	5.41 (3.96)	0.023*
PHE	1.77 (2.49)	3.39 (2.61)	0.673	2.08 (2.51)	1.89 (2.62)	0.857	3.74 (1.87)	1.81 (2.53)	0.062
ANT	N/A (4.43)	3.95 (4.65)	0.335	N/A (4.52)	N/A (4.68)	0.810	4.33 (4.67)	N/A (4.52)	0.041*

NAP - Naphthalene, ACE - Acenaphthene, ACY – Acenaphthylene, FLO – Fluorene, PHE – Phenanthrene, ANT – Anthracene; *Sig. difference $p < 0.05$; N/A: Not available (the result was not available for target compounds due to the smallest number)

Table 4.5.2: Median (IQR) of association between the home environment factors and concentration of LMW-PAHs detected in blood serum (ng/mL) by using Mann-Whitney U Test

Target compounds	Exposure to traffic congestion			Open Burning activities			Passive smokers			Existence of highway		
	Yes (n=73)	No (n=123)	p-Value	Yes (n=141)	No (n=52)	p-Value	Yes (n=81)	No (n=116)	p-Value	Yes (n=89)	No (n=109)	p-Value
NAP	11.24 (5.66)	5.11 (1.86)	<0.001*	5.29 (2.28)	11.16 (6.38)	<0.001*	5.33 (4.68)	6.60 (6.11)	0.411	11.16 (6.11)	5.04 (1.39)	<0.001*
ACE	2.76 (1.69)	1.54 (2.27)	<0.001*	1.65 (2.22)	2.50 (3.59)	<0.001*	1.89 (2.48)	1.97 (2.48)	0.434	2.63 (1.92)	N/A (2.14)	<0.001*
ACY	12.30 (8.15)	9.61 (1.95)	<0.001*	9.74 (2.18)	13.74 (10.88)	<0.001*	9.74 (2.88)	10.24 (6.43)	0.279	13.61 (8.49)	9.52 (1.65)	<0.001*
FLO	8.30 (4.15)	5.05 (1.51)	<0.001*	5.15 (1.89)	8.30 (5.84)	<0.001*	5.42 (2.66)	5.62 (4.10)	0.531	8.37 (4.18)	4.90 (1.31)	<0.001*
PHE	3.85 (0.44)	1.55 (0.80)	<0.001*	1.73 (2.35)	3.85 (0.41)	<0.001*	1.77 (2.49)	3.48 (2.32)	0.025*	3.85 (0.40)	1.47 (0.66)	<0.001*
ANT	4.49 (0.56)	N/A	<0.001*	N/A (4.22)	4.47 (0.48)	<0.001*	N/A (4.47)	4.06 (4.49)	0.072	4.53 (0.60)	N/A	<0.001*

NAP - Naphthalene, ACE - Acenaphthene, ACY – Acenaphthylene, FLO – Fluorene, PHE – Phenanthrene, ANT – Anthracene; *Sig. difference $p < 0.05$; N/A: Not available (the result was not available for target compounds due to the smallest number)

Table 4.5.3 Median (IQR) of association between the lifestyles factor and concentration of LMW-PAHs detected in blood serum (ng/mL) by using Mann-Whitney U Test and Kruskal-Wallis Test

Target Compounds	Diet (grilled food)			Cooking Mode (indoor grilling and frying)			Hobbies			Transportation Mode					
	Yes (n=163)	No (n=33)	p-value	Yes (n=162)	No (n=34)	p-value	Outdoor ^a (n= 151)	Indoor ^b (n=21)	p-value	Car (n=68)	Motor (n=66)	Train (n=1)	Bus (n=24)	Walk (n=29)	p-value
NAP	6.40 (6.15)	5.19 (1.15)	0.051	6.21 (6.02)	5.02 (2.48)	0.091	5.64 (3.84)	9.34 (6.46)	0.066	5.69 (5.53)	5.16 (2.19)	N/A	10.80 (7.91)	6.77 (6.76)	<0.001*
ACE	1.97 (2.73)	0.77 (2.17)	0.024*	1.80 (2.59)	1.98 (2.37)	0.865	1.79 (2.49)	2.43 (3.43)	0.123	1.57 (2.40)	N/A (2.27)	N/A	2.81 (1.74)	2.08 (2.84)	<0.001*
ACY	10.13 (5.39)	9.66 (2.26)	0.798	10.17 (4.99)	9.45 (2.31)	0.126	10.04 (3.38)	9.73 (6.90)	0.770	9.74 (2.97)	9.55 (1.80)	N/A	13.61 (8.01)	11.40 (7.48)	0.016*
FLO	5.68 (4.07)	4.76 (0.10)	0.013*	5.40 (4.00)	5.51 (1.98)	0.292	5.18 (3.48)	6.25 (3.92)	0.015*	5.05 (2.81)	5.00 (1.65)	N/A	8.91 (4.29)	6.10 (3.07)	<0.001*
PHE	2.08 (2.52)	1.76 (0.98)	0.070	2.06 (2.50)	1.69 (1.98)	0.360	1.83 (2.49)	3.74 (2.20)	0.033*	1.80 (2.41)	1.54 (0.98)	N/A	3.92 (0.40)	3.75 (2.42)	<0.001*
ANT	N/A (4.59)	N/A	0.011*	N/A (4.55)	N/A (3.25)	0.267	N/A (4.44)	4.37 (4.76)	0.019*	N/A (4.32)	N/A	N/A	4.67 (0.73)	4.34 (4.77)	<0.001*

NAP - Naphthalene, ACE - Acenaphthene, ACY – Acenaphthylene, FLO – Fluorene, PHE – Phenanthrene, ANT – Anthracene; *Sig. difference $p < 0.05$; a: Outdoor Sports and Gardening, b: Indoor Sports, N/A: Not available (the result was not available for target compounds due to the smallest number)

CHAPTER 5

DISCUSSION

5.1 Concentration of LMW-PAHs

Based on the results in Table 4.4, there were significant differences between the concentration LMW-PAHs of the exposed and comparative group where the p -value less than 0.05. Next, the highest median concentrations in both exposed and comparative group were acenaphthylene, the concentrations in exposed and comparative group were 13.44 ng/mL and 9.45 ng/mL respectively. Lucadamo et al. (2021) stated that there was a significant association between the spatial variation of diesel vehicle rates and those of acenaphthylene, acenaphthene, fluorene, anthracene, and naphthalene.

5.2 Association between contributing factors and concentration of LMW-PAHs

5.2.1 Work environment factors

Results in Table 4.5.1 showed that there was a significant different between the exposure to chemical in working environment and three of the target compounds (naphthalene, fluorene and anthracene). According to the self-reported exposure information in the questionnaire, there were several respondents working in

manufacturing company that produced the pest repellent. The occupational exposure of the pest repellent could lead to naphthalene deposition in their body. Naphthalene is an ubiquitous pollutant, sometimes encountered indoors when this chemical is used as a pest repellent or deodorant (Batterman et al., 2012).

5.2.2 Home environment factors

Based on the results in Table 4.5.2, majority of home environment factors were significantly associated with the LMW-PAHs in blood serums. Based on the result from table 4.5.2, there were association between all compound of LMW-PAHs and traffic congestion as most of the respondents were exposed to traffic congestion based on the survey from the questionnaire. Previous study reported one of the main sources of polycyclic aromatic hydrocarbons (PAHs) is vehicle exhaust, and roadside soils (Kim et al., 2019). Besides, recent years have shown an increase amount of PAHs deposited in the roadside soil of Dhanbad due to ineffective public transportation, traffic congestion, and incomplete fuel combustion (Suman et al., 2016). Therefore, the exposure to traffic congestion was suggested as one of the main contributing factors to the deposition of the LMW-PAHs in the human body in this study. Next, there were also significant association between the open burning activities and LMW-PAHs in blood serums. It has been founded that the highest concentrations of naphthalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[a]anthracene, and chrysene are produced during the simulated combustion of a variety of natural and synthetic materials used to assess the risks associated with forest fires, landfill fires, and household waste incineration (Świt et al., 2022).

5.2.3 Lifestyles factors

Results in Table 7 showed that the association between the lifestyle factor and concentration of LMW-PAHs in blood serums. Firstly, there was no significant association between the cooking mode (with and without indoor grilling and frying) and concentration of LMW-PAHs. Most of the previous studies stated that from cooking activities with high temperatures such as deep-frying methods generate more high molecular weight PAHs (HMW-PAHs) which are benzo[a]pyrene (B[a]P) because they consume greater volumes of edible oil and involve higher oil temperatures relative to those of frying methods (Yao et al., 2015; Hao et al., 2016). Most of the researchers reported LMW-PAHs not the major contributor from indoor cooking activities (grilling and frying) (Rose et al., 2015). Hence, cooking mode (indoor grilling and frying) was not the major contributing factors towards LMW-PAHs in blood serum of human.

Furthermore, there were significant differences between the transportation mode and concentration of LMW-PAHs in blood serums. This showed that the transportation mode was one of the crucial contributing factors for the exposure of PAHs in human blood. According to research from Abdel-Shafy and Mansour (2016), petrogenic PAHs are common due to the widespread transportation, storage, and use of crude oil and crude oil products. There were also studies that stated both gaseous and granular kinds of PAHs can be emitted during vehicle operation. Numerous studies have shown that traffic-related emissions from PAHs make up 35% of all atmospheric emissions, and that the concentration of PAHs in the air along a traffic line is between 5.3 and 8.3 times greater than the average for the atmospheres of urban and rural areas, respectively (Cui et al., 2022).

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This chapter will conclude the study, which will summarise the major findings in relation to the objectives and research questions and analyse their importance and contribution. Additionally, it will discuss the study's limitations and suggest possibilities for additional investigation. This study aimed to determine the occurrence of LMW-PAHs in low and high traffic volume area in Klang Valley, Selangor and its contributing factors of exposure. The results indicate that the concentration of LMW-PAHs was significantly higher in blood serums of exposed group compared to the comparative group. Further findings show that, open burning activities, traffic congestion, existence of highway and the mode of transportation to commute daily were major contributing factors for the concentrations of LMW-PAHs in blood serums.

Previously, there were limited studies that focus on the specific molecular weight compounds of PAHs as most of them were focus on the general PAHs. This study will be a baseline for the future research to do more investigation regarding to specific target compounds especially on the health risk assessment due to exposure of low molecular weight of PAHs in the environment. From this study, there were recall bias when the respondents need to answer the questionnaire by recalled their memory about daily routines. To overcome this limitation in the future, the questionnaire given to the respondent can be also guided by the family members and guided by researchers.

6.2 Recommendations

The occurrence of LMW-PAHs in low and high traffic volume area can be high especially when they are influenced by the contributing factors. The exposure of LMW-PAHs will indicate a high risk to human health. Thus, more researches are recommended to investigate the health risk of PAHs among the exposed population especially on the cancerous effects and DNA changes.

In addition, there are recommendations for the communities to control the exposure of PAHs. The precaution includes practicing car-pooling or using the public transport. Next, avoid the residential area near highway or heavy traffic. After that, avoid from open burning activities and practice other technique to eliminate the waste such as practice waste segregation, 3R (recycle, reuse and reduce), food waste compost. Small effort can give a huge impact to maintain the sustainability of the environment.

6.3 Study limitations

Based on the research that have been carried out, there were limited evidences that can be referred for the association of low molecular weight of PAHs in blood serums and different contributing factors. Next, the collected data having issues such as missing data from respondents as the respondent missed answered certain questions in the questionnaire and the respondents' exposure information was self-reported.

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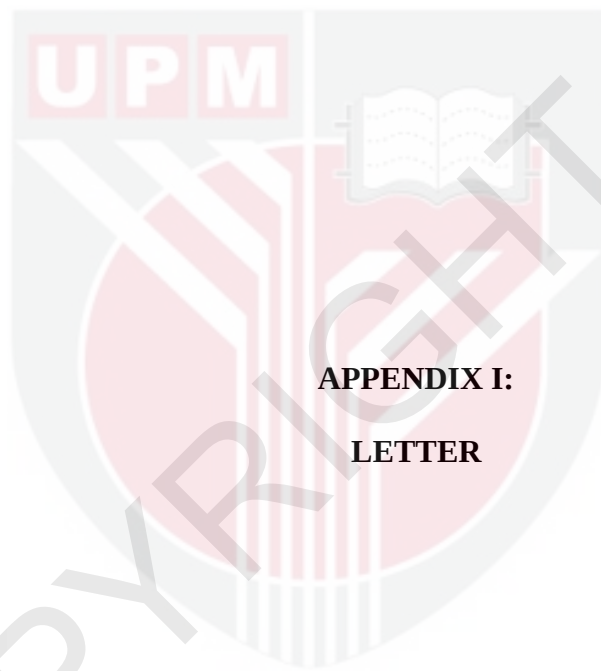
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APPENDIX I:
LETTER



UPM
UNIVERSITI PUTRA MALAYSIA



PEJABAT TIMBALAN NAIB CANSOLOR (PENYELIDIKAN DAN INOVASI)
OFFICE OF THE DEPUTY VICE CHANCELLOR (RESEARCH AND INNOVATION)

Reff. : UPM/TNCPI/RMC/1.4.18.2 (JKEUPM)
Date : 18 February 2022

Assoc. Prof. Dr. Ho Yu Bin
Department of Environmental and Occupational Health
Faculty of Medical Science and Health Sciences
43400 UPM Serdang, Selangor

Dear Sir/ Madam,

PROJECT TITLE: ASSOCIATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN AIR WITH LEUKOCYTE TELOMERE LENGTH AMONG ADULT POPULATION LIVING IN HIGH AND LOW TRAFFIC VOLUME AREAS IN MALAYSIA

JKEUPM REF. NO: JKEUPM-2018-285

PRINCIPAL INVESTIGATOR: ASSOC. PROF. DR. HO YU BIN

STUDENT: SAMER SAAD HAMEED AL-BATTAWI

Your document received on 23 January 2022 is referred.

The committee has approved your request to extend the duration of study for another year, starting from 18 February 2022 until 18 February 2023.

Thank you.

"WITH KNOWLEDGE WE SERVE"

Yours faithfully,

PROF. DR. ZAMBERI SEKAWI

Chair
Ethics Committee for Research Involving Human Subjects
Universiti Putra Malaysia

Bil	Nama penyelidik	Tugas penyelidik dalam projek
1.	Prof. Madya Dr. Ho Yu Bin	Penyelidik Utama/Penyelia
2.	Dr. Samer Saad Hameed Al-Battawi	Penyelidik Siswazah
3.	Puan Amrina Mohd Amin	Pegawai Hematologi
4.	Puan Zamzarina Hj Ahmad Bajari	Pegawai Hematologi
5.	Puan Nor Saldatul Badariah Ismail	Pegawai Hematologi
6.	Nurul Haziqah Binti Abdullah	Pelajar Tahun Akhir Prasiswazah
7.	Siti Maryam Binti Che Ruslay	Pelajar Tahun Akhir Prasiswazah
8.	Alna Basyirah Binti Mustapa Kamal	Pelajar Tahun Akhir Prasiswazah

5. Penyertaan pihak tuan dalam kajian ini adalah sangat saya hargai dan segala kerjasama dari pihak tuan saya dahului dengan ucapan ribuan terima kasih.

Sekian,

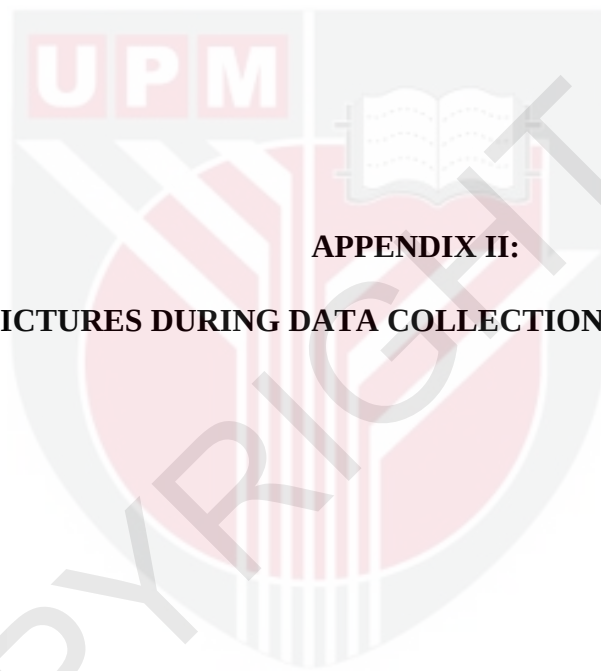
"BERILMU BERBAKTI"

Yang menjalankan tugas,



PROF. MADYA DR. HO YU BIN
Jabatan Kesihatan Persekitaran dan Pekerjaan
Fakulti Perubatan dan Sains Kesihatan
Universiti Putra Malaysia

UPM



APPENDIX II:

PICTURES DURING DATA COLLECTION AND ANALYSIS



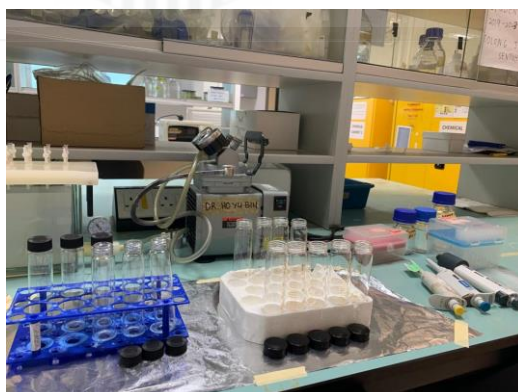
Pictures above shows the data collection process



The collected blood samples were kept in ice box



The samples were centrifuged to obtain the blood serum



Apparatus used during extraction analysis



The process of vortexing



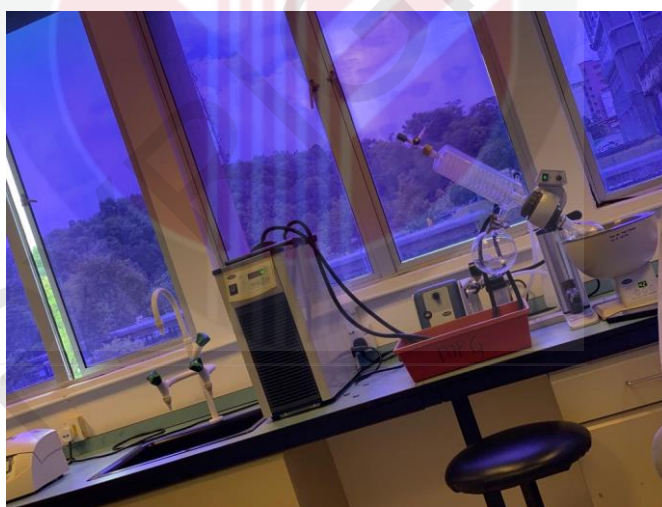
The process of sonication



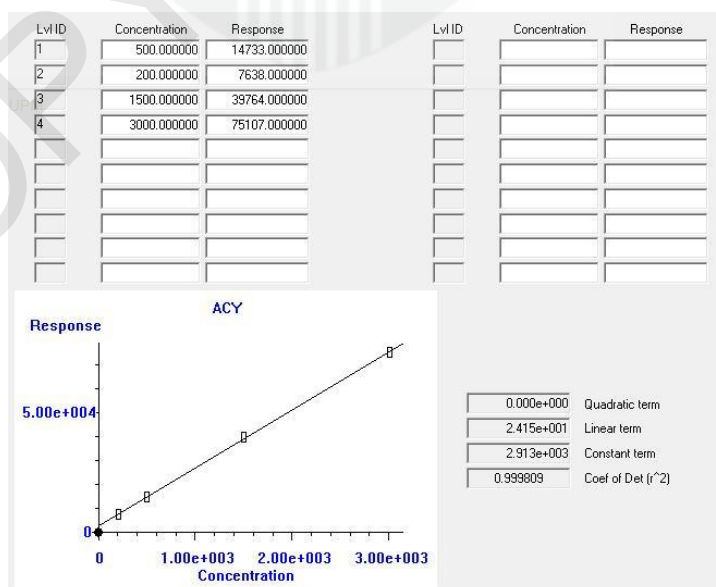
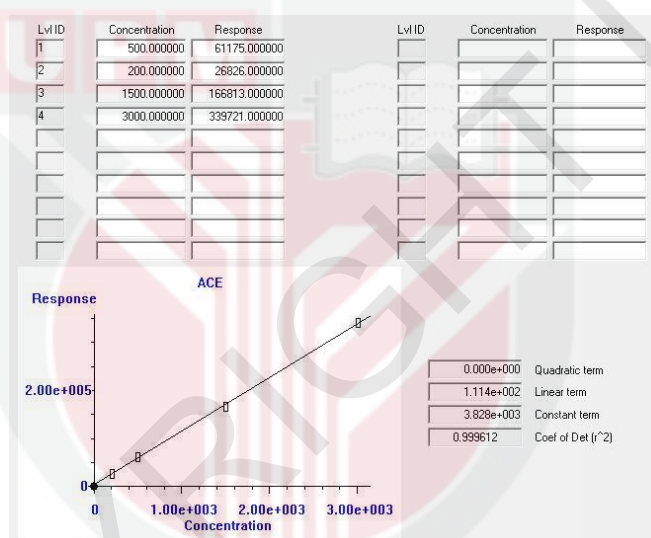
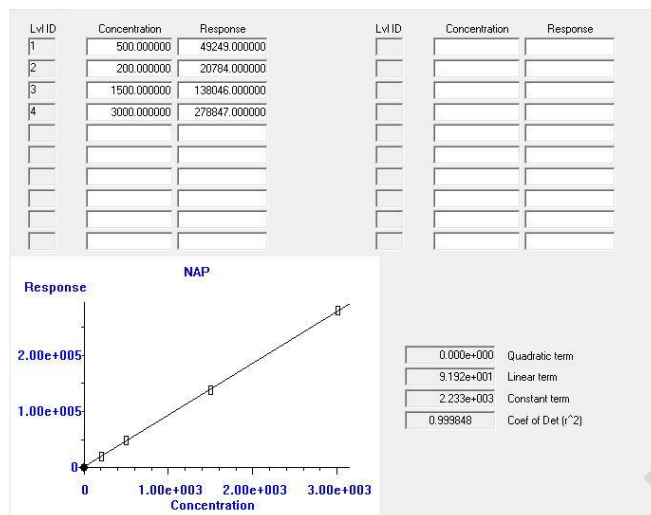
Centrifugation process



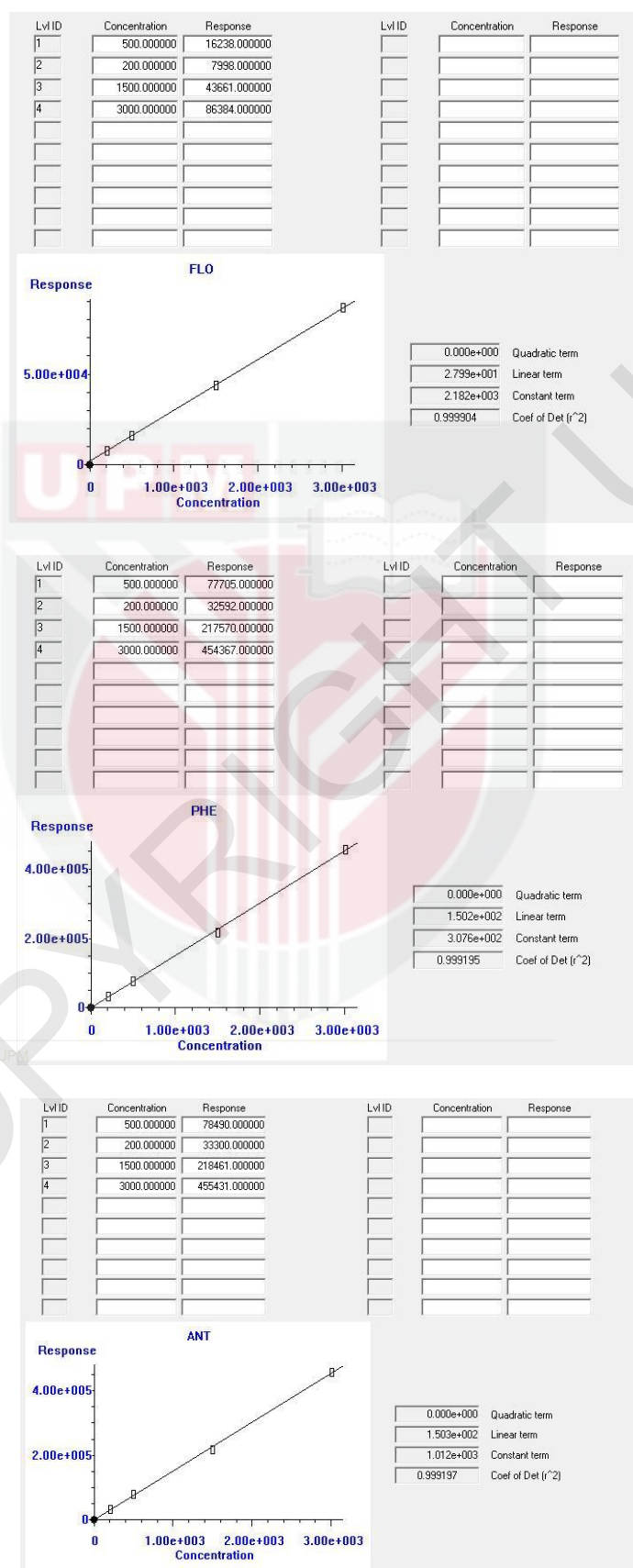
cleaned-up with solid phase extraction (SPE)



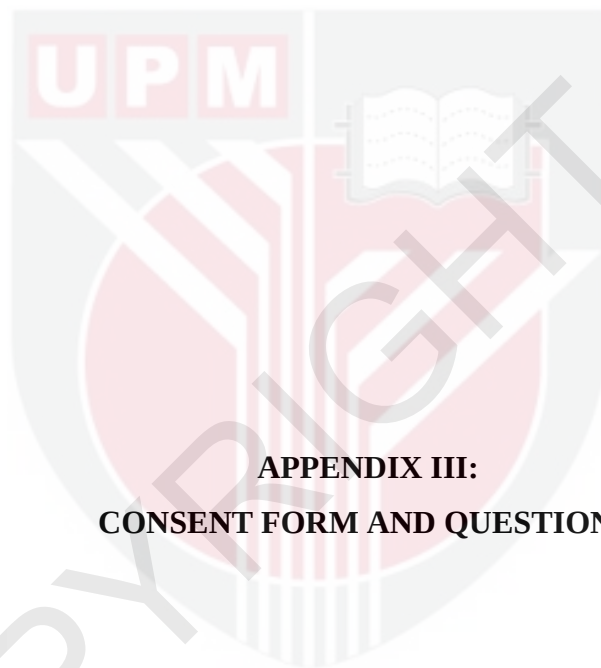
Rotary evaporator used to reduce the volume of combined extract



Picture above shows the calibration curve result from GC-MS Analysis



Picture above shows the calibration curve result from GC-MS Analysis



**APPENDIX III:
CONSENT FORM AND QUESTIONNAIRES**



BORANG 2.4 PENERANGAN DAN PERSETUJUAN RESPONDEN

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

1. TAJUK KAJIAN

Hubungan polisiklik aromatik hidrokarbon dengan panjang telomer leukosit antara penduduk dewasa yang tinggal di kawasan trafik yang tinggi dan rendah di Malaysia.

2. PENGENALAN

Polisiklik aromatik hidrokarbon (PAHs) adalah campuran sebatian organik yang dilepaskan ke atmosfera sebagai gas atau zarah semasa pembakaran bahan organik yang tidak lengkap. Walau bagaimanapun, pelepasan asap kenderaan dijangka menjadi sumber PAHs yang paling utama memandangkan kenderaan bermotor menyumbang sebanyak 73.8% bahan pencemar udara di Malaysia. Kajian terkini tentang panjang telomer dan pendedahan kepada bahan pencemar udara yang berkaitan dengan lalu lintas yang tinggi dalam kalangan orang dewasa yang sihat telah menunjukkan terdapat pemendakan telomer yang dikaitkan dengan peningkatan kadar pencemaran udara. Sehingga kini, tiada kajian mengkaji hubungan antara PAH dan telomer yang berkaitan dengan lalu lintas di kalangan penduduk dewasa di Malaysia. Objektif kajian ini adalah untuk mengkaji PAH yang terdapat di PM_{2.5} di dalam udara, kandungan PAH dalam serum darah dan panjang telomer leukosit (LTL) dalam kalangan penduduk dewasa yang tinggal di kawasan trafik yang tinggi (HTV) dan terendah (LTV) di Selangor. Seramai 296 responden yang terdiri daripada 148 orang dewasa dari HTV (kumpulan terdedah) dan 148 orang dewasa dari LTV (kumpulan tidak terdedah) akan mengambil bahagian dalam kajian ini.

Telomer: Merujuk kepada susunan Asid deoksiribonukleik (DNA) yang berulang dan terletak pada hujung kromosom yang melindungi bahagian hujung kromosom (Schwab, 2011).

3. APAKAH YANG PERLU ANDA LAKUKAN?

Pensampelan udara menggunakan high volume air sampler akan dilakukan di kawasan kajian selama 3 hari berturut-turut untuk mengukur tahap PAHs. Responden dikehendaki untuk mengambil bahagian dalam temubual soal selidik dan pengambilan sampel darah di Klinik Desa Batu 18 di Hulu Langat, dan di Klinik Prime Care di Petaling Jaya, Selangor. Pengambilan sampel darah akan dilakukan oleh kakitangan perubatan yang sah di klinik-klinik ini mengikut prosedur yang ditetapkan untuk mengukur kandungan residu PAH dalam darah dan panjang telomer. Responden dikehendaki untuk pergi ke klinik ini bagi mengambil sampel darah. Responden perlu mengambil bahagian dalam temu bual soal selidik untuk mendapatkan maklumat berkenaan dengan maklumat peribadi, pendedahan kepada PAH, status pemakanan dan status kesihatan. Temubual soal selidik akan mengambil masa kira-kira 10 minit untuk setiap sesi. Mereka yang telah menyelesaikan semua temubual soal selidik dan pengambilan akan diberikan RM50.00 sebagai tanda penghargaan untuk menyertai kajian ini.

4. SIAPA YANG TIDAK BOLEH MENYERTAI KAJIAN INI?

Perokok dan pengambil minuman beralkohol tidak boleh menyertai kajian ini. Mereka telah didiagnos dengan hipertensi, diabetes mellitus, HIV/AIDS, dan kanser juga tidak boleh mengambil bahagian dalam kajian ini. Individu yang berumur di bawah 18 tahun dan 55 tahun keatas juga tidak dibenarkan mengambil bahagian dalam kajian ini. Penyertaan akan dibatalkan jika responden mempunyai pendedahan kepada PAH di tempat kerja.

5. APAKAH FAEDAH MENYERTAI KAJIAN INI?

a) KEPADA ANDA SEBAGAI PESERTA?

Kajian ini akan memberikan idea kepada responden mengenai kandungan PAH di udara dan dalam darah mereka serta panjang telomer mereka. Sekiranya responden ingin mengetahui hasil kajian, mereka akan dimaklumkan oleh para penyelidik melalui surat berdaftar selepas kajian selesai. Melalui hasil kajian ini, satu program perkongsian pengetahuan yang komprehensif akan dilakukan dalam kalangan masyarakat yang tinggal di kawasan trafik yang tinggi tentang risiko pendedahan kepada PAHs dan promosi gaya hidup sihat.

b) KEPADA PENYELIDIK?

Melalui kajian ini, kandungan PAH dalam udara dan serum darah serta panjang telomer responden akan ditentukan. Hubungan antara kandungan PAH dalam udara, darah dan panjang telomer juga akan dikenalpasti. Kami juga boleh memberikan maklumat kepada penggubal dasar dan polisi mengenai kandungan PAH yang terdapat di udara dan kesan kesihatan kepada penduduk bandar. Pembuat dasar boleh dimaklumkan tentang pentingnya mempromosikan pengangkutan mesra alam, kepentingan meningkatkan pengangkutan awam dan menghadkan bilangan kenderaan pada suatu masa di satu kawasan untuk mengurangkan pencemaran udara.

6. ADAKAH IA BERISIKO?

Responden yang mengambil bahagian dalam kajian ini mungkin akan menghadapi risiko kesakitan, atau ketidakselesaan semasa pengambilan darah. Terdapat juga risiko jangkitan, lebat dan bengkak di kawasan pengambilan darah. Untuk memastikan risiko dikurangkan sebanyak mungkin, pengambilan darah akan dilakukan oleh kakitangan perubatan yang sah dari klinik swasta mengikut prosedur yang ditetapkan. Penyertaan responden dalam kajian ini adalah secara sukarela dan tidak akan dipaksa. Setiap responden mempunyai hak untuk menarik diri dari kajian ini pada mana-mana peringkat penyertaan tanpa sebarang penalti.

7. ADAKAH MAKLUMAT DAN IDENTITI SAYA KEKAL RAHSIA?

Ya. Maklumat dan identiti responden akan kekal rahsia sepanjang kajian dijalankan dan selepas kajian. Semua maklumat dan rekod adalah sulit dan tidak akan dikongsi kepada orang lain Pemantau, auditor, panel pemantau etika JKEUPM dan pihak berkuasa mempunyai akses kepada maklumat dan rekod kesihatan responden hanya untuk tujuan kajian sahaja. Maklumat genetik yang didapati melalui kajian ini tidak akan didedahkan kepada sesiapa termasuk ahli keluarga tanpa persetujuan responden. Rekod kesihatan dan sampel darah yang semasa kajian ini akan digunakan hanya untuk tujuan kajian dan akan dilupuskan dengan cara yang sesuai selepas kajian ini tamat.

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

Kajian ini telah diluluskan oleh Panel Kajian Etika JKEUPM dan boleh dicapai melalui hubungan berikut untuk maklumat mengenai hak peserta kajian, termasuk keluhan dan aduan.

PUSAT PENGURUSAN PENYELIDIKAN
Bangunan Pej, Timbalan Naib Canselor (Penyelidikan dan Inovasi)
Tingkat 4 & 5 Universiti Putra Malaysia
43400 UPM Serdang, Selangor

No. Telefon: 0389471601
Faks: 0389451596
Emel: dir.rm@upm.edu.my

Sekiranya terdapat sebarang pertanyaan lain, sila hubungi nombor dan emel seperti di bawah:

Ketua Penyelidik: Dr. Ho Yu Bin
No. Telefon: 012-6140221
Emel: yubin@upm.edu.my

Penyelidik: Samer Saad Hameed Al-Battawi
No. Telefon: 018-2439527
Emel: dr_samlr87@yahoo.com

Sila tanda tangan di sini sekiranya anda telah membaca dan memahami kandungan halaman ini

9. PERSETUJUAN

Saya No Kad Pengenalan
beralamat
.....dengan ini bersetuju untuk mengambil bahagian secara sukarela
dalam penyelidikan yang tersebut di atas "(kajian klinikal/ percubaan ubat-ubatan/
rakaman video/ kumpulan sasaran/ temuduga/ soal selidik).

Saya telah diberi penjelasan secara menyeluruh mengenai penyelidikan ini dari segi metodologi, risiko dan komplikasi (seperti tertulis pada Helatan Penerangan Responden). Saya memahami bahawa saya berhak menarik diri dari penyelidikan ini pada bila-bila masa tanpa memberi sebarang alasan. Saya juga memahami bahawa sebarang maklumat yang berkaitan identiti saya akan dirahsiakan.

Saya* berminat / tidak berminat untuk mengetahui keputusan kajian yang melibatkan saya.

I setuju/tidak bersetuju untuk emel/gambar/rakaman video/ rakaman suara digunakan dalam apa jua bentuk penerbitan atau pembentangan. (sekiranya berkaitan).

*potong yang tidak berkenaan

Tandatangan Tandatangan
(Responden) (Saksi)

Tarikh : Nama :

No. K/P :

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

Tarikh : Tandatangan
(Penyelidik)



**JABATAN KESIHATAN PERSEKITARAN DAN PEKERJAN
FAKULTI PERUBATAN DAN SAINS KESIHATAN UNIVERSITI
PUTRA MALAYSIA**

**PERBANDINGAN BERAT MOLEKUL RENDAH POLISIKLIK
AROMATIK HIDROKARBON DALAM SAMPEL SERUM DARAH
DALAM KALANGAN PENDUDUK DEWASA YANG TINGGAL DI
KAWASAN TRAFIK YANG TINGGI DAN RENDAH DI SELANGOR**

SOALAN UTAMA

Soal selidik ini terdiri daripada Empat (4) bahagian:

Bahagian A: Maklumat peribadi

Bahagian B: Pendedahan kepada polisiklik aromatic hidrokarbon

Bahagian C: Status pemakanan

Bahagian D: Status kesihatan

**PERBANDINGAN BERAT MOLEKUL RENDAH POLISIKLIK AROMATIK
HIDROKARBON DALAM SAMPEL SERUM DARAH DALAM KALANGAN
PENDUDUK DEWASA YANG TINGGAL DI KAWASAN TRAFIK YANG TINGGI
DAN RENDAH DI SELANGOR**

BAHAGIAN A: MAKLUMAT PERIBADI

1. Jantina: Lelaki Perempuan

2. Bangsa: Melayu Cina India

☐☐☐

Lain-lain (Sila nyatakan):

3. Perkahwinan status: Bujang ☐ Berkahwin ☐

Bercerai/ Ibu/ Bapa tunggal ☐

4. Bilangan Anak:

5. Tahun Pendidikan:

• Tiada Pendidikan formal

☐

• UPSR

☐

• PMR/ SPM

☐

• Diploma, STPM, Matrikulasi

☐

• Sarjana Muda, Sarjana, PhD

☐

**BAHAGIAN B: PENDEDAHAN KEPADA POLISIKLİK AROMATİK
HIDROKARBON**

6. Adakah terdapat perokok lain di rumah anda? Jika "Ya", sila jawab soalan 8 dan 9.
Jika "Tidak", sila jawab soalan 10.

☐ Ya ☐ Tidak

7. Berapa banyak perokok yang terdapat di rumah selain anda?

☐ 1 ☐ 2 ☐ 3 ☐ >3

8. Apakah jenis rokok yang dihisap oleh perokok lain?

☐ Rokok

☐ Vapes

☐ Shisha

☐ Lain-lain (Sila nyatakan):

9. Apakah hobi anda pada masa lapang?

☐ Berkebun ☐ Sukan Luaran

☐ Sukan Dalam ☐ Lain-lain (Sila nyatakan):

10. Berapa jam yang anda gunakan untuk perjalanan setiap hari?

Jam

11. Apakah jenis pengangkutan yang anda gunakan untuk perjalanan setiap hari?

☐

Kereta

☐

Motosikal

☐

Komuter

☐

Bas

☐

Berjalan kaki

12. Adakah anda bekerja di luar atau dalam bangunan?

☐

Luar

☐

Dalam

13. Berapa jamkah anda bekerja setiap hari?

Jam

14. Pernahkah anda bekerja dalam persekitaran yang terdedah kepada bahan kimia, gas atau asap yang berbahaya?

☐

Ya

☐

Tidak

15. Pekerjaan sekarang:

16. Bilangan tahun yang anda bekerja di tempat sekarang Tahun

17. Bilangan hari berkerja setiap minggu hari seminggu

18. Adakah terdapat kesesakan lalu lintas berhampiran kediaman anda?

☐

Ya

☐

Tidak

19. Adakah terdapat lebuh raya berhampiran kediaman anda?

☐

Ya

☐

Tidak

20. Adakah anda makan daging ayam, ikan atau makanan laut panggang? Jika "Ya", sila nyatakan berapa hidangan anda makan setiap minggu?

☐

Ya

☐

Hidangan/ minggu

☐

Tidak

21. Adakah terdapat sebarang aktiviti memanggang atau menggoreng di dalam rumah anda? Jika "Ya", sila nyatakan berapa kali aktiviti tersebut dijalankan di dalam rumah anda setiap minggu?

Ya

Kali / minggu

Tidak

22. Adakah terdapat sebarang aktiviti pembakaran di rumah anda? Jika "Ya", sila jawab soalan 23. Jika "Tidak", sila jawab soalan 24.

Ya

Tidak

23. Apakah jenis pembakaran di rumah anda?

Kayu/arang/daun

Sampah

Gegelung nyamuk

Lain-lain (sila nyatakan): _____

24. Berapa jam yang anda gunakan di rumah anda setiap hari?

BAHAGIAN C: STATUS PEMAKANAN

25. Berapa banyak cawan sayuran berdaun hijau mentah yang anda makan setiap hari?

*1/2 cawan = 125 ml

☐ Tiada pengambilan

☐ 1/2 cawan

☐ 1 cawan

☐ ≥ 2 cawan

26. Berapa banyak cawan sayuran berdaun hijau yang dimasak (contohnya brokoli, bayam, atau lobak merah) adakah anda makan setiap hari?

☐ Tiada pengambilan

☐ 1/2 cawan

☐ 1 cawan

☐ ≥ 2 cawan

27. Berapa cawan cendawan masak yang anda makan setiap hari?

☐

Tiada pengambilan

☐

1/2 cawan

☐

1 cawan

☐

≥ 2 cawan

28. Berapa banyak cawan buah-buahan (contohnya, pisang, oren, atau epal) yang anda makan setiap hari?

☐

Tiada pengambilan

☐

1/2 cawan

☐

1 cawan

☐

≥ 2 cawan

29. Adakah anda mengambil multivitamin atau suplemen? Jika "Ya", sila jawab Soalan 29

☐

Ya

☐

Tidak

30. Berapa lamakah anda telah mengambil multivitamin atau suplemen?

☐

Bulan

☐

Tahun

~ Soal selidik berakhir ~

Terima kasih atas kerjasama anda

Q/ Adakah anda ingin mengetahui hasil kajian ini? Jika ya, sila berikan butiran yang diperlukan.

☐

Ya, email: _____

☐

Tidak

Q/ Apakah hari dan masa pilihan anda untuk pengambilan darah? Silatandakan hari dan masa pilihan anda.

Isnin	
Selasa	
Rabu	
Khamis	
Jumaat	
Sabtu	
Ahad	

09 am - 11 am	
10 am - 12 pm	
11 am - 01 pm	
12 pm - 02 pm	
01 pm - 03 pm	



**JABATAN KESIHATAN PERSEKITARAN DAN
PEKERJAN FAKULTI PERUBATAN DAN SAINS
KESIHATAN UNIVERSITI PUTRA MALAYSIA**

**PERBANDINGAN BERAT MOLEKUL RENDAH
POLISIKLİK AROMATİK HIDROKARBON DALAM
SAMPEL SERUM DARAH DALAM KALANGAN PENDUDUK
DEWASA YANG TINGGAL DI KAWASAN TRAFİK YANG
TINGGI DAN RENDAH DI SELANGOR**

SOALAN SARINGAN

Nama Responden	
Nombor IC	
Nombor Telefon	

**PERBANDINGAN BERAT MOLEKUL RENDAH POLISIKLIK AROMATIK
HIDROKARBON DALAM SAMPEL SERUM DARAH DALAM KALANGAN
PENDUDUK DEWASA YANG TINGGAL DI KAWASAN TRAFIK YANG TINGGI
DAN RENDAH DI SELANGOR**

Kriteria Kemasukan		Ya	Tidak
Umur	18-55 tahun		
Kawasan tinggal & berkerja	Adakah anda bekerja dan tinggal di kawasan yang sama sekurang-kurangnya 3 bulan?		

Kriteria pengecualian		Ya	Tidak
Merokok	1. Adakah anda merokok lebih daripada 100 batang rokok sepanjang hayat anda?		
	2. Adakah anda merokok dalam tempoh 28 hari yang lepas?		
Alkohol	1. Berapa kerapkah anda mengambil minuman yang mengandungi alkohol? A. Tidak pernah (0 Markah) B. Sekali sebulan atau kurang (1 Markah) C. 2-4 kali sebulan (2 Markah) D. 2-3 kali seminggu (3 Markah) E. ≥ 4 kali seminggu (4 Markah)	Markah	
	2. Pada hari yang anda mengambil minuman beralkohol, berapa banyak minuman yang anda ambil? *(1 minuman = 1 tin bir = 1 gelas wain = 1 shot spirit) A. 1 atau 2 minuman (0 Markah) B. 3 atau 4 minuman (1 Markah) C. 5 atau 6 minuman (2 Markah) D. 7 atau 8 atau 9 minuman (3 Markah) E. ≥ 10 minuman (4 Markah)	Markah	
	3. Berapa kerapkah anda mengambil enam atau lebih minuman beralkohol dalam satu masa? A. Tidak pernah (0 Markah) B. Kurang daripada setiap bulan (1 Markah) C. Setiap bulan (2 Markah) D. Setiap minggu (3 Markah) E. Setiap hari (4 Markah)	Markah	
	Jumlah skor soalan (1-3) * Responden layak menyertai kajian ini jika jumlah skor adalah (0-2)	3-12	0-2

Kriteria pengecualian		Ya	Tidak
Asma	1. Pernahkah anda mempunyai asma? Jika Ya, sila terus ke soalan 2 dan 3.		
	2. Adakah ia disahkan oleh doktor perubatan?		
	3. Adakah anda mengambil sebarang ubat untuk asma?		
Hipertensi (tekanan darah tinggi)	1. Adakah anda mempunyai tekanan darah tinggi? Jika Ya, sila terus ke soalan 2 dan 3.		
	2. Adakah ia disahkan oleh doktor perubatan?		
	3. Adakah anda mengambil sebarang ubat untuk tekanan darah tinggi ?		
Diabetes Mellitus	1. Adakah anda mempunyai kandungan glukosa darah yang tinggi? Jika Ya, sila terus ke soalan 2 dan 3.		
	2. Adakah ia disahkan oleh doktor perubatan?		
	3. Adakah anda mengambil sebarang ubat untuk diabetes mellitus?		
HIV/ AIDS	1. Adakah anda mempunyai penyakit HIV/ AIDS?		
	2. Adakah ia disahkan oleh doktor perubatan?		
Kanser	1. Adakah anda menghidapi kanser?		
	2. Adakah ia disahkan oleh doktor perubatan?		
Pendedahan Industri ke PAHs	1. Adakah anda sedang bekerja di salah satu industri berikut? - Penghasilan aluminium primer - Penghasilan kok - Industri kimia - Penghasilan tayar getah - Penghasilan simen - Industri asfalt		

~ Soal selidik berakhir ~

Terima kasih atas kerjasama anda, responden yang memenuhi criteria akan dihubungi untuk menyertai kajian ini.