



UNIVERSITI PUTRA MALAYSIA

***DISTRIBUTION AND CONCENTRATION OF LINEAR ALKYL BENZENES
IN RIVER SEDIMENTS AND SHORT-NECK CLAMS (*Paphia Undulata*) IN
PENINSULAR MALAYSIA***

SAMI MUHSEN SLEMAN MAGAM

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By

SAMI MUHSEN SLEMAN MAGAM

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
Fulfilment of the Requirements for Degree of Doctor of Philosophy**

March 2018

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DEDICATION

To the sources of my inspiration:

The memory of my beloved mother;

My beloved father;

My beloved wife;

My lovely daughters (Malak and Farah);

All my brothers and sisters;

All other family members and respected friends

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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SAMI MUHSEN SLEMAN MAGAM

March 2018

Chairperson: Normala bt. Halimoon, PhD
Faculty: Environmental Studies

Due to population growth and extensive urbanization in recent years, urban wastewater has entered the rivers and coastal environment in Peninsular Malaysia, causing risks to human health and posing threats to other organisms. Therefore, tracing regional anthropogenic influences is important for assessing the magnitude of pollution caused by human activities and its consequences to the environment, especially aquatic ecosystems. In the present study, the presence of linear alkylbenzenes (LABs) that can be used as sawage indicator in aquatic environment was examined in sediments and short-neck clams (*Paphia Undulata*) tissue. This study aimed to investigate the distribution, concentration and composition of LABs, and also degradation of LABs with the emphasis on the role of bioavailability of LABs to *P. undulata* from sediments and dissipating LABs from input sources to remote areas. Sediment samples were collected from selected rivers and estuaries from the Kedah, Merbok, Prai, Perak, Klang Melaka, Segget, Kuantan, Terengganu, and Kelantan, while *P. undulata* samples were taken from the Kedah, Merbok, Prai, Perak, Klang and Melaka Estuaries in Peninsular Malaysia from January 2013 to February 2014. The sediments and *P. undulata* samples were homogenized, freeze-dried, extracted, cleanup, fractionated and analyzed using gas chromatography mass spectrometry (GC-MS). The concentrations of total LABs in sediments varied from 55.22 to 3117.16 ng g⁻¹ dry weight (dw) and *P. undulata* varied from 21.09 to 861.65 ng g⁻¹ dw. LABs in sediments can be classified as low to moderate (55.22 to 313.69 ng g⁻¹) contamination in the Kedah, Kuantan, Perlis, Merbok, Kelantan, Terengganu and Perak, moderate to high (977.81 to 1,427.37 ng g⁻¹) in the Segget, and Melaka and high to very high (2,909.59 to 3,117.16 ng g⁻¹) in the Prai and Klang Rivers and Estuaries. LABs in the soft tissues of *P. undulata* can be classified as moderate contamination in the Kedah and Melaka, low contamination in the Perak and Merbok and high contamination in the Prai and Klang. The compositional profiles of LABs in sediments and *P. undulata* of the studied areas demonstrated that the levels of long chain alkylbenzenes (C13; 33 %) were higher than short chain alkylbenzenes (C10; 11 %). The results from the ratio of internal (I) and external (E) isomers of LABs (I/E ratios), in the surface sediments were from 0.56

to 3.18, while the results from I/E ratios in *P. undulata* were from 0.70 to 3.21 indicating a range of raw, primary to secondary treated sewage effluents. The values of the ratio of 5-C₁₃LAB+5-C₁₂LAB relative to 5-C₁₁LAB+5-C₁₀LAB (L/S ratio) ranged from 1.04 to 4.03 in the sediments and from 0.87 to 4.87 in (*P. undulata*). Meanwhile, the values of the ratio of 6-,5-,4-,3-and 2-C₁₃/6-, 5-, 4-, 3-, and 2-C₁₂LAB (C₁₃/C₁₂ ratio) ranged from 1.14 to 3.54 in the sediments and from 2.23 to 4.78 in *P. undulata* in the coastal areas of Peninsular Malaysia. These values are slightly higher than those of the commercial detergents. There was significant correlation ($r = 94$, $p < 0.05$) between total parent LABs in sediments and in *P. undulata* which indicated the ability of *P. undulata* to accumulate the LABs. Therefore, *P. undulata* can be considered as a good biomonitor of LABs in the aquatic environment. Based on the results from the LABs concentrations, it is recommended that different sites of Malaysia should be monitored and managed to reduce the levels of LABs entering the aquatic environment.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**TABURAN DAN KEPEKATAN ALKILBENZENA LURUS DI DALAM
SEDIMENT SUNGAI DAN KERANG BERLEHER PENDEK (*Paphia
Undulata*) DI PENINSULAR MALAYSIA**

Oleh

SAMI MUHSEN SLEMAN MAGAM

March 2018

Pengerusi: Normala bt. Halimoon, PhD

Fakulti: Pengajian Alam Sekitar

Disebabkan pertumbuhan populasi dan perbandaran yang meluas, air sisa perbandaran telah mengalir ke sungai dan pantai di Semenanjung Malaysia menyebabkan risiko kepada kesihatan manusia dan ancaman kepada organisma-organisma lain. Oleh itu, pengesanan pengaruh antropogenik serantau juga adalah penting untuk menilai tahap pencemaran yang disebabkan oleh aktiviti-aktiviti manusia dan kesan-kesannya terhadap alam sekitar terutamanya ekosistem akuatik. Dalam kajian ini, penggunaan alkilbenzena lurus (LABs) yang boleh digunakan sebagai penunjuk kumbahan di persekitaran akuatik diperiksa di sedimen dan tisu leher pendek (*Paphia Undulata*). Kajian ini bertujuan untuk untuk menyasat taburan, kepekatan dan komposisi LABs, dan juga degradasi LABs dengan penekanan terhadap peranan keupayaan biologi LABs ke atas (*P. undulata*) dari sedimen-sedimen dan pengaliran LABs dari sumber masuk ke kawasan terpencil. Sampel-sampel sedimen diambil dari sungai-sungai dan muara-muara terpilih di Kedah, Merbok, Prai, Perak, Klang Melaka, Segget, Kuantan, Terengganu, dan Kelantan, manakala kerang berleher pendek (*P. undulata*) telah diambil dari Kedah, Merbok, Prai, Perak, Klang dan muara Melaka di Semenanjung Malaysia dari Januari 2013 hingga Februari 2014. Sampel-sampel sedimen dan *P. undulata* telah dihomogen, dibekukering, diekstrak, dibersihkan, difraksinasi dan dianalisa menggunakan kromatografi gas spektrometri jisim (GC-MS). Jumlah kepekatan LAB dalam sedimen adalah dari 55.22 hingga 3,117.16 ng g⁻¹ berat kering (dw) dan dalam *P. undulata* adalah dari 21.09 ke 861.65 ng g⁻¹ dw. LAB dalam sedimen-sedimen boleh diklasifikasikan berpencemaran rendah ke sederhana (55.22 hingga 313.69 ng g⁻¹) di Kedah, Kuantan, Perlis, Merbok, Kelantan, Terengganu dan Perak, sederhana ke tinggi (977.81 hingga 1,427.37 ng g⁻¹) di Segget dan Melaka dan tinggi ke sangat tinggi (2,909.59 hingga 3,117.16 ng g⁻¹) di sungai dan muara Prai dan Klang. LABs di dalam tisu lembut *P. Undulata* boleh dikelaskan sebagai berpencemaran sederhana di Kedah dan Melaka, berpencemaran rendah di Perak dan Merbok serta berpencemaran tinggi di Prai dan Klang. Profil komposisi LABs dalam sedimen-sedimen dan *P. undulata* di kawasan kajian menunjukkan bahawa alkilbenzena rantai panjang (C13; 33%) adalah lebih tinggi daripada alkilbenzena rantai pendek (C10; 11%). Keputusan daripada nisbah dalaman per

luaran (I/E) di permukaan sedimen adalah dari 0.56 ke 3.18, manakala keputusan daripada nisbah I/E dalam *P. undulata* adalah dari 0.70 ke 3.21 menunjukkan suatu julat efluen kumbahan mentah, efluen kumbahan terawat primer ke sekunder. Nilai nisbah 5-C₁₃LAB+5-C₁₂LAB terhadap 5-C₁₁LAB+5-C₁₀LAB (nisbah L/S) adalah ber julat antara 1.04 hingga 4.03 di dalam sedimen-sedimen dan antara 0.87 to 4.87 di dalam *P. undulata*. Sementara itu, nilai nisbah 6-,5-,4-,3-dan 2-C₁₃ / 6-, 5-, 4-, 3-, dan 2-C₁₂LAB (nisbah C₁₃/C₁₂) adalah ber julat antara 1.14 hingga 3.54 di dalam sedimen-sedimen dan antara 2.23 hingga 4.78 di dalam *P. undulata* di kawasan pantai Semenanjung Malaysia. Nilai-nilai ini adalah sedikit tinggi daripada nilai yang terdapat di dalam detergen-detergen komersial. Terdapat korelasi yang signifikan ($r = 94, p < 0.05$) antara jumlah LABs induk di dalam sedimen-sedimen dan di dalam *P. undulata* ($p < 0.05$) yang menunjukkan keupayaan *P. undulata* mengumpul LABs di persekitaran akuatik. Oleh itu, *P. undulata* boleh dijadikan sebagai biomonitor LABs yang baik dalam persekitaran akuatik. Berdasarkan keputusan dari kepekatan-kepekatan LABs, adalah disyorkan agar kawasan-kawasan berlainan di Malaysia harus dipantau dan diuruskan untuk mengurangkan paras kemasukan LABs ke persekitaran akuatik.

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LIST OF ABBREVIATIONS

LABs	Linear alkyl benzenes
LASs	Linear alkyl benzene sulfonates
TABs	Tetra propylene-based alkylbenzenes
TBS	Tetra propylene benzene sulfonates
TAMs	Trialkylamines
LOD	Limit of detection
LOQ	Limit of quantification
PAHs	Polycyclic Aromatic Hydrocarbons
POPs	Persistent organic pollutant
PCBs	Polychlorinated biphenyls
ABS	Alkylbenzene sulfonates
a-TA	a-Tocopheryl acetate
APR	Aminopropanone
BASs	Branched alkylbenzene sulphonates
Biphe-d10	Biphenyl-deuterated -10
DATs	Dialkyltetralinsulfonates
K _{wo}	Octanol – water partition coefficient
TOC	Total organic carbon
GC-MS	Gas chromatography-Mass spectrometry
I/E	a ratio of 6-,5-C ₁₂ LAB relative to 4-,3-,2-C ₁₂ LAB
L/S	a ratio of 5-C ₁₃ LAB+5-C ₁₂ LAB relative to 5-C ₁₁ LAB+5-C ₁₀ LAB
C ₁₃ /C ₁₂	a ratio of 6-,5-,4-,3-and 2-C ₁₃ / 6-, 5-, 4-, 3-, and 2-C ₁₂ LAB
LC-LABs	Longer chains alkylbenzenes
SC-LABs	Shorter chains alkylbenzenes
WWTP	wastewater treatment plants
SPM	suspended particulate matters
SIS	Surrogate Internal Standard
IIS	Internal Injection Standard
DCM	Dichloromethane
Hex	Hexane
MeOH	Methanol
IWK	Indah Water Konsortium Sdn Bhd
UKM	Universiti Kebangsaan Malaysia
SCS	South China Sea
Silicones	Polyorganosiloxanes
5-C ₁₀	5-phenyldecane
4-C ₁₀	4-phenyldecane

3-C ₁₀	3-phenyldecane
2-C ₁₀	2-phenyldecane
6-C ₁₁	6-phenylundecane
5-C ₁₁	5-phenylundecane
4-C ₁₁	4-phenylundecane
3-C ₁₁	3-phenylundecane
2-C ₁₁	2-phenylundecane
6-C ₁₂	6-phenyldodecane
5-C ₁₂	5-phenyldodecane
4-C ₁₂	4-phenyldodecane
3-C ₁₂	3-phenyldodecane
2-C ₁₂	2-phenyldodecane
7,6-C ₁₃	7, 6-phenyltridecane
5-C ₁₃	5-phenyltridecane
4-C ₁₃	4-phenyltridecane
3-C ₁₃	3-phenyltridecane
2-C ₁₃	2-phenyltridecane
7-C ₁₄	7-phenyltetradecane
6-C ₁₄	6-phenyltetradecane
5-C ₁₄	5-phenyltetradecane
4-C ₁₄	4-phenyltetradecane
3-C ₁₄	3-phenyltetradecane
2-C ₁₄	2-phenyltetradecane

CHAPTER 1

INTRODUCTION

1.1 Background of study

In the last few decades, the rapidly-growing population, industrialization, and urbanization in the world have been accompanied by growing health risks due to the increasing inputs of wastes, including untreated domestic wastewater, into the environment, especially the aquatic environments (UN, 2003). As a matter of fact, most of the environmental degradation issues are attributed to human activities such as lodging, industrial activities, and waste disposal (Shahbazi et al., 2010). Specifically, the lack of precautionary steps taken by humans when engaged in such activities is the main reason behind these environmental problems. Efficiency of the municipal sewage treatment systems can decrease the load of pollutants in the sewage discharged directly into the aquatic environment. However, in most of the rural areas the sewage treatment service is not available and inefficient if available (Alkhadher et al., 2016). Subsequently, organic wastes associated with the untreated sewage effluents are discharged into the aquatic environment in many developing countries where the proper facilities for collection and treatment of sewage are still lacking (Takada et al., 1992; Martins et al., 2008).

Malaysia is situated in Southeast Asia, surrounded by the South China Sea and the Strait of Melaka. Its land area borders with Thailand in the north while its maritime areas border with Indonesia, Singapore, and Vietnam. The population of Malaysia was estimated at 30 million in the year of 2014 (Department of Statistics Malaysia, 2014). The climate of Peninsular Malaysia is characterized by heavy rainfall as is common for a tropical rainforest climate.

Rapid urbanization and industrialization in Malaysia over the recent decades have resulted in an increase in the use of the household detergents and the release of municipal and industrial effluents and domestic wastes, which are reasons for serious concern about safety and health of the Malaysian aquatic environment (Alkhadher et al., 2015). These detergents and wastes led to an increase in the levels of the linear alkylbenzens (LABs) in the local environment. In recent years, urban and industrial development also increased in line with the increase in the population of Malaysia (Masood et al., 2016). Consequently, the environment of Malaysia is nowadays receiving a multitude of hazardous compounds from various anthropogenic sources (Tsutsumi et al., 2002; Isobe et al., 2004). The different wastes have industrial pollutants, which is a factor that contributed to an increase in the industrial pollution, including the release of surfactants such as the LABs into the aquatic environment (Rinawati et al. (2013). These compounds are released from anthropogenic sources such as the industrial activities and, as a group, are one of the persistent organic pollutants (POPs).

The Malaysian rivers and estuaries are constantly exposed to various types of pollutants. The pollution problems have drawn intense public attention with the increasing industrialization and urbanization in Malaysia since the 1980s and the sources of land and sea-based pollution in the Malaysian aquatic environment have been overviewed (Shahbazi et al., 2010). Huge threats are posed on the aquatic environment in Malaysia by domestic discharge, industrial and agricultural effluents, and sewage contamination. A significant threat is particularly imposed on the aquatic environment by the wide use of *synthetic detergents in urban areas and industries*. In fact, pollutants associated with sewage are released into the aquatic environment through spills or leaks of household detergents, municipal and industrial effluents, and domestic waste (Takada et al., 1992). The rapidly-growing population and concomitant increase in the use of detergents result in release of a wide array of pollutants, including LABs, into the aquatic ecosystem. The LABs derive from industrial and domestic wastes and are transported to the aquatic environment through lateral transport, urban runoff, and drainage, among others (Rinawati et al., 2012). In addition, a large amount of untreated sewage enters the coastal environment when the storm water drainage systems and the sewage systems are combined as a result of rainstorm overloads. This seriously pollutes the waterways and surrounding areas (Isobe et al., 2004).

Earlier research (Tsutsumi et al., 2002) has shown that the origins and distribution of LABs in Malaysia differ across locations. For example, in the west coast of Peninsular Malaysia, the high urban development and intense industrial activity introduce LABs into the aquatic environment from non-point sources, including urban run-off, and point sources like factories. Nevertheless, there is still limited information on the environmental distribution of LABs in Malaysia (Isobe et al., 2004). Therefore, it is an obligation to investigate the amounts and sources of the LABs that are released into the aquatic environment.

Molecular markers are extremely useful for detection of sewage and domestic waste and identification of the sources and transport pathways of sewage in the aquatic environment (Takada and Eganhouse, 1998; Eganhouse, 2004; Dwiwitno et al., 2016). In this context, the LABs are a group of the most useful markers for tracing organic contaminants from point and non-point sources of pollution due to their stability in sediments and biodegradation during transport. Such molecular markers of sewage pollution as the LABs can be utilized to detect of sewage pollution in Malaysian rivers (Tsutsumi et al., 2002; Isobe et al., 2007; Alkhadher et al., 2015). Consequently, this study investigates the levels and distribution of LABs in the Malaysian aquatic environment.

1.2 Problem statement

The rivers and estuaries in the urban, industrial, and agricultural areas of Peninsular Malaysia are contaminated to various degrees with sewage (Isobe et al., 2004). The rivers under study (i.e., Perlis, Kedah, Merbok, Perai, Perak, Klang, Melaka, Segget, Kuantan, Terengganu, and Kelantan rivers) are surrounded by fast-growing industries and urban centers, which impose high health risks to the public and the riverine environment through sewage discharges. Thus, monitoring LABs contaminants as sewage indicators is very important.

Huge amounts of untreated wastewater or poorly-treated sewage are being discharged into the aquatic ecosystems of Peninsular Malaysia. Stranded or remobilized LABs attached to suspended particulates and sediments may also become available to filter feeders, particularly clams that inhabit the inter-tidal and shallow sub-tidal seabed. One of these filter feeder organisms is *Paphia undulata* (*P. undulata*), which is an important protein source in West Malaysia. However, it can sequester many lipophilic organic compounds such as the LABs and it can, thus, be a good biomonitor of these contaminants. At present, no information is available regarding the concentrations of LABs in short-neck clam or carpet clam (*P. undulata*) originating from the rivers and estuaries of Peninsular Malaysia. In consequence, this study aims at investigating the concentrations, distribution, and possible sources of LABs that are accumulated by short-neck clams (*P. undulata*) in this region. LABs are a suite of chemical markers that have been successfully utilized as organic molecular markers for evaluating the source of sewage pollution (Eganhouse, 1997). Due to their exclusive association with human activities, LABs can be used as evidence of anthropogenic impact on the marine environment. Most of LABs production is used to manufacture linear alkylbenzene sulfonates (LAS), which is used in household synthetic detergents production (Eganhouse et al., 1983). Because of this, LABs are fast becoming the most common raw material in the manufacturing of household detergents. Due to its improved biodegradability and cost-effectiveness, LABs have completely replaced the older branched alkylbenzene in the production of surfactants that have been used in household laundry detergents and dishwashing applications since the 1960s.

Environmental protection against release of hazardous materials is a major scientific, social, and economic purpose of the world today. Contamination of coastal areas can be looked upon as a serious threat to human health and natural resources. In this regard, clear understanding of the distribution and sources of LABs in the environment is the first step to control and reduce the negative impacts of these contaminants on the aquatic environment and public health. In this respect, investigation of contamination with the LABs has been carried out in various locations across the globe during recent years (Heim et al., 2004; Hartmann et al., 2005). However, this issue has not been well-addressed in the aquatic ecosystems of Peninsular Malaysia so far and, hence; further studies need to be performed in order to develop a better understanding of the problem. To the researcher's best knowledge, this is the first study investigating the levels and distribution of LABs in all the major rivers in Peninsular Malaysia.

1.3 Significance of the study

One of the most significant environmental concerns in the third millennium is safeguarding water resources, including the aquatic ecosystem (World Bank, 2010). Therefore, scrutinizing the natural waterways and implementing suitable management of wastewater for emerging contaminants have become unavoidable actions to ensure good quality of water and a healthy ecosystem. This study evaluated the levels of LABs in different rivers and estuaries in Peninsular Malaysia and compared between the rivers in these parameters. This study is significant since it generated valuable data on the concentrations and sources of LABs in the riverine and estuarine sediments of selected rivers all over Peninsular Malaysia (i.e., Perlis, Kedah, Merbok, Perai, Perak, Klang, Melaka, Segget, Kuantan, Terengganu, and Kelantan rivers). The study provides

assessment of the extent of pollution with LABs in these rivers. Moreover, in order to efficiently control release of LABs into the environment, the present study provides data on the sources of LABs in the studied rivers.

On the other hand, there are some reported studies that evaluated the LAB concentrations in aquatic organisms such as green mussel (*Perna viridis*) (e.g., Tsutsumi et al. (2002) and Isobe et al. (2007)). The bivalves can be good biomonitor for contaminants like the LABs because of their sessile living style, high abundance, ease of culture in the laboratory, and convenient sampling. They occupy an important position in the food chain and can tolerate a high amount of contaminants without dying. Within this context, short-neck clams (*P. undulata*) is considered as an important protein source in West Malaysia. It has the ability to sequester many lipophilic organic compounds such as LABs and can be a good biomonitor. The different species of LABs may have different bioavailabilities for short-neck clams (*P. undulata*) and only the bioavailable fractions of the contaminants can pass through the food chain. Hence, this study is significant in that it evaluated suitability of short-neck clams (*P. undulata*) as sentinel biomonitor of LAB pollution was also evaluated.

Fine materials like clay, sand, and shell fragments in the water column may act as sorptive materials for organic contaminants (Martins et al., 2010) and all kinds of pollution stemming from human activities and reaching to surface water systems will eventually settle down in surface sediments (Abdullah et al., 1999; Vaezzadeh et al., 2014; Keshavarzifard, et al., 2015), which constitute the sink for the organic contaminants that are strongly associated with the suspended particulate matter. In this respect, most of the contaminants demonstrate their fingerprints in the sediments and, therefore, sediment analysis has certain advantages over the analysis of water. Thus, the study of LABs in sediments is of high importance due to the high affinity of LABs with sewage and suspended particles. Owing to that the sedimentary ecosystems are exposed to the accumulation of LABs in the sediments because of the increasing pollution level along the rivers and estuaries (Alkhadher et al., 2016), the results of this study will help in specifying whether or not the sediment ecosystem in Malaysia is affected by sewage pollution by using LABs as indicator markers.

1.4 The objectives of the study

The main objective of this study was to analyze the amount of pollution of the surface sediments and tissues of *P. undulata* with LABs originating from anthropogenic sources. The specific objectives of the study were:

- I. To determine the concentrations, distribution and compositions of LABs in sediments and short-neck clams (*P. undulata*) tissues in the selected rivers around Peninsular Malaysia.
- II. To identify the degree of LABs degradation in the sediments and short-neck clams (*P. undulata*) tissues in Peninsular Malaysia using diagnostic indices.
- III. To investigate the relationships between the concentrations of LABs in sediments and tissues of short-neck clams (*P. undulata*) in the studied areas.

1.5 Hypotheses

This study initiated based upon the hypotheses as listed below:

- i. There is no significant difference between the mean concentrations of LABs in the sediment and tissues of *P. undulata* in the rivers of Peninsular Malaysia due to similar source of pollution.
- ii. Raw and primary sewage effluents are the major sources of LABs in sediments and tissues of *P. undulata* due to inadequate and/or improper treatment plants in the study area.
- iii. *P. undulata* is capable of accumulation of LABs in tissues due to hydrophobic characteristics of LABs.

1.6 Research questions

The major questions which this research sought to provide answers for were:

- (1) What are the distributions, concentrations, and compositions of LABs in the sediments and tissues of short-neck clams (*P. undulata*) in the studied rivers and estuaries?
- (2) To what extent were the LABs biodegraded in the aquatic environment?
- (3) Are there significant correlations between the levels of the investigated LABs in the sediments and total organic carbon (TOC) in the studied rivers?
- (4) What are the strengths and directions of the correlation between LABs in sediments and tissues of short-neck clams (*P. undulata*) in the studied rivers?

1.7 Thesis structure

The thesis structure is a simply flow chart describing the thesis design contents. The first chapter is an Introduction to the study. It provides background on the study, the research problem, significance of the study, and the research questions and objectives. The second chapter is the literature review chapter. It provides a comprehensive review of previous related research on distribution, sources, pathways, and fate of organic contaminants in the environment across the globe, with emphasis on LABs and on Peninsular Malaysia.

The third chapter is the methodology chapter. It describes the study area, sampling locations, sample preparation, and sample analysis. The fourth chapter presents and discusses the study results. The first, second, and third sections of this chapter present the results related to the distribution and sources of LABs in the sediments. Thus, these three sections cover the first research objective. The fourth, fifth, and sixth sections of the chapter discuss the results pertaining to the distribution and sources of LABs in short-neck clams (*P. undulata*), thus addressing the second research objective.

Finally, the fifth chapter highlights the main conclusions of this study and gives recommendations for future research.

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