# EXPOSURE TO ENDOTOXIN LEVEL IN INHALABLE RICE DUST AMONG RICE MILL WORKERS

By

ROS AIDA BINTI MOHD ZIN

Dissertation submitted in partial fulfillment of the requirement for the degree of Bachelor of Health Sciences (Biomedicine)

MAY 2016

# EXPOSURE TO ENDOTOXIN LEVEL IN INHALABLE RICE DUST AMONG RICE MILL WORKERS

By

ROS AIDA BINTI MOHD ZIN

Dissertation submitted in partial fulfillment of the requirement for the degree of Bachelor of Health Sciences (Biomedicine)

MAY 2016

## ACKNOWLEDGEMENT

I express my deep gratitude to the Almighty Allah who created and nurture this transitory world for giving me an opportunity to do this final year project under the School of Health Sciences.

It is a genuine pleasure to express my sense of thanks and gratitude to my supervisor and co-supervisor, Dr. Sabreena Safuan and Dr. Siti Marwanis Anua for their dedication, guidance, valuable advice and keen interest above all overwhelming attitude to help me for completing my final year project. Their meticulous scrutiny, endless inspiration, encouragement, scholarly advice and scientific approach have helped me to a very great extent to accomplish this project.

I express my deep and sincere gratitude to Mrs Tan Chian Lee, Manager of Kilang Padi Liangtong (K.B.) Sdn. Bhd. whose encouragement, suggestion, kindness, allowed me to collect the dust samples.

I gratefully acknowledge the cooperation of all participating workers. I express my sincere thanks to my family for emotional and financial support to finish this final year project. Not to forget to all those who have helped to make this study possible, in particular the staffs of the Biomedicine laboratory, Environment and Occupation Health laboratory and my fellow friends. This study is funded under USM's RUI grant (research university individue) 1001/PPSK/812181.

## TABLES OF CONTENTS

## LIST OF TABLES

**Page**

## LIST OF FIGURES

**Page**

## LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMS

| | |
|---|---|
| % | Percentage |
| µL | Microlitre |
| µm | Micrometre |
| °C | Degree celcius |
| B | Average mass change of blanks (mg) |
| C | Airborne aerosol concentration |
| CAD | Coronary artery disease |
| CI | Confidence interval |
| COPD | Chronic obstructive pulmonary disease |
| df | Degrees of freedom |
| EU/ml | Endotoxin units per milliliter |
| FEV1 | Forced expiratory volume in one second |
| FVC | Forced vital capacity |
| g | Gram |
| GFA | Glass fiber filter |
| IOM | Institute of Occupational Medicine |
| IQR | Interquartile range |
| KAP | Knowledge, attitude and practices |
| LAL | *Limulus amebocyte lysate* |
| LBP | Lipopolysaccharide binding protein |
| LFT | Lung function test |

| | |
|---|---|
| LPSs | Lipid portions of lipopolysaccharides |
| $M_1$ | Mass of filter (plus cassette where used) before sampling (mg) |
| $M_2$ | Mass of filter (plus cassette where used) after sampling (mg) |
| $m^3$ | Cubic metre |
| mg | Milligram |
| $mg/m^3$ | Milligram per cubic metre |
| MHC | Major histocompatibility complex |
| mL | Millilitre |
| mm | Millimetre |
| MODS | Multiple organ dysfunction syndromes |
| MT | Metric ton |
| nm | Nanometre |
| NO | Nitric oxide |
| PBST | Phosphate buffer saline with Tween-20 |
| PC | Polycarbonate |
| ppm | Parts per million |
| PPE | Personal protective equipment |
| SIRS | Systemic inflammatory response syndrome |
| SOP | Standard operating procedure |
| $t\ ha^{-1}$ | 1 tonne per hectare |
| TNF-α | Tumor necrosis factor alpha |
| VC | Vital capacity |
| $V_S$ | Volume of air sampled ($m^3$) |

## ABSTRAK

Endotoksin adalah lipid dalam lipopolisakarida (LPS) yang terdiri daripada membran luar dinding sel bakteria gram-negatif. Habuk beras yang mengandungi endotoksin boleh menyebabkan sindrom respons inflamasi sistemik (SIRS) dan sindrom disfungsi organ pelbagai (MODS). Matlamat dan objektif kajian ini adalah untuk membandingkan kepekatan habuk, tahap pendedahan endotoksin dan fungsi paru-paru antara pekerja kilang beras dan pekerja pengurusan di Kelantan. Sampel telah dikumpul dengan menggunakan alat penapis Institut Perubatan Pekerjaan (IOM) selama 8 jam dan dianalisis menggunakan ujian Limulus lisat amebosit (LAL). Fungsi paru-paru telah diuji dengan menggunakan spirometer untuk mengenalpasti jumlah maksimum udara semasa menarik nafas sebelum pendedahan dan selepas pendedahan kepada habuk. Hasil kajian menunjukkan bahawa kepekatan habuk ($mg/m^3$) pekerja kilang beras (median: 8.86, julat: 2.68-15.93) adalah berbeza berbanding pekerja pengurusan USM (median: 1.47, julat: 0.22-2.53) dan pekerja pengurusan kilang beras (median: 0.70, julat: 0.47-1.72) $p$=0.005 (<0.05). Kepekatan endotoksin (EU/ml) pekerja kilang beras (median: 0.14, julat: 0.11-0.16) adalah berbeza berbanding pekerja pengurusan kilang beras (median: 0.06, julat: 0.03-0.10) dan pekerja pengurusan USM (median: 0.05, julat: 0.04-0.09). Kapasiti vital paksa (FVC), isipadu ekspirasi paksa (FEV1) dan FEV1 / FVC% dalam kalangan tiga kumpulan menunjukkan tiada perbezaan yang signifikan sebelum dan selepas pendedahan ($p$=0,601, $p$=0,960 dan $p$=0,660), masing-masing. Kajian ini penting untuk meningkatkan kesedaran kesihatan di kalangan pekerja dari segi pengetahuan, sikap dan amalan keselamatan peralatan perlindungan peribadi (PPE) serta dapat memberi manfaat kepada penyelidik lain untuk menyiasat lebih lanjut tentang debu beras di Malaysia.

## ABSTRACT

Endotoxins are the lipid portions of lipopolysaccharides (LPSs) which is a part of the outer membrane of the cell wall of gram-negative bacteria. Rice dust containing endotoxin can cause systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndromes (MODS). The aim and objective of this study are to compare the concentration of dust, endotoxin exposure level and lung function between rice mill workers and management workers in Kelantan. Samples were collected using Institute of Occupational Medicine (IOM) inhalable dust sampler loaded with glass fiber filter (GFA) for 8 hours and analysed by using Limulus amebocyte lysate (LAL) test. The lung functions were tested by using the spirometer to measure the amount of maximal air breathed out over pre-exposure (before work) and post-exposure (after work). Results showed that the dust concentration ($mg/m^3$) of rice mill workers (median: 8.86, range: 2.68-15.93) was significantly different compared with the USM management workers (median: 1.47, range: 0.22-2.53) and the rice mill management workers (median: 0.70, range: 0.47-1.72) $p$=0.005. The endotoxin concentration (EU/ml) of the rice mill workers (median: 0.14, range: 0.11-0.16) was significantly different compared with the rice mill management workers (median: 0.06, range: 0.03-0.10) and the USM management workers (median: 0.05, range: 0.04-0.09). The forced vital capacity (FVC), forced expiratory volume in one second (FEV1) and FEV1/FVC% among the three groups shows no different between pre and post-exposure at ($p$=0.601, $p$=0.960 and $p$=0.660), respectively. This study is important to promote health awareness among workers involving knowledge, attitude and safety practices about personal protective equipment (PPE) and gives benefit for other researchers for further investigations about rice dust in Malaysia.

# CHAPTER 1

# INTRODUCTION

## 1.1 Background of study

Rice, which consists of carbohydrate, protein, minerals and enzymes, is a major part of everyday Malaysian diet. Rice or also called *Oryza sativa L.* of Gramineae family has wide geographical distribution and long history of cultivation in Asian countries. These have led to the development of a great diversity of varietal types of rice (Bardenas & Chang, 1965). The first cultivation of rice was in South and Southeast Asia and the first puddle and transplant of rice was developed in north and central China (Chang, 1976). Nowadays, the cultivation of rice was carried out in more than hundred countries in the world. As population increases, demand for rice also increases. Rice industries expanded and a lot of workers are needed. In addition, the occupational disease will also increase. Rice dust is the major occupational health risk that can cause respiratory illnesses and overt lung disease among the rice mill workers

Endotoxins are the lipid portions of lipopolysaccharides (LPSs) which is a part of the outer membrane of the cell wall of gram-negative bacteria (Caroff & Karibian, 2003; Erridge et al., 2002). Endotoxins are release when the immune system or antibiotics attack the bacteria and breaks down their cell walls (Agelaki et al., 2002; Holzheimer, 2001; Lepper et al., 2002; Prins et al., 1995). When the cell walls broken, fragments of the outer membrane are released into the body. Endotoxins caused the released of histamine,

interleukin-1 (IL-1) and interleukin-2 (IL-2), tumor necrosis factor (TNF), activation of coagulation system and bradykinin (Miller & Keane, 2003). These molecules can cause allergic reactions, dilating blood vessels, provoke diarrhea, headache, congestion of the nose, asthmatoid wheezing, hypotension, arrhythmia, urticaria, pruritus and flushing (Maintz & Novak, 2007).

Exposure to endotoxins in a large quantity can cause hemorrhagic shock and severe diarrhea while in small amount; endotoxins produce fever, altered resistance to bacterial infection, leukopenia followed by leucocytosis and numerous other biological effects (Miller & Keane, 2003). Endotoxins are significant problems among workers that can cause respiratory illnesses and lung disease. According to Todar (2008), endotoxin can cause fever, inflammation, changes in the white blood cell counts, disseminated intravascular coagulation, hypotension, shock and death. Endotoxins are less potent and less specific in their action compare with exotoxin, since they do not act enzymatically, heat stable and cannot be converted to bacterial toxins or toxoids (Todar, 2008).

Rice dust containing endotoxin can cause systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndrome (MODS). According to Neviere et al. (2012), SIRS is a form of dysregulated inflammation involving sepsis, autoimmune disorder, pancreatitis, vasculitis, thromboembolism or burn. MODS is classified as primary or secondary that refers to progressive organ dysfunction in an acute patient (Neviere et al., 2012). Silverman and Ostro (1999) stated that systemic infections (septicemias) are a source of endotoxin exposure.

Limited and incomplete data have been documented on the endotoxin exposure level especially among the rice mill workers exposed to rice dust which is reported to have high silica content in its structure. A review of the literature also failed to find any data on the state of knowledge, attitude and practices (KAP) with regards to workplace dust exposure in Malaysia especially rice husk dust (Musa, 2006). There were hardly any data in the Western Pacific region about the effectiveness of workplace intervention programs.

## 1.2 Objectives of study

### *1.2.1 General objective:*

To determine endotoxin exposure level of inhalable rice dust among rice mill workers and its effects in lung function.

### *1.2.2 Specific objectives:*

1. To determine the level of dust exposure among rice mill workers and management workers.
2. To determine the level of endotoxin exposure among rice mill workers and management workers.
3. To determine the lung functions among rice mill workers and management workers.
4. To compare the dust exposure level between rice mill workers and management workers.
5. To compare the endotoxin exposure level between rice mill workers and management workers.

6. To compare lung functions between rice mill workers and management workers.

## 1.3 Hypothesis

### *1.3.1 Hypothesis 1*

1. Null hypothesis:

   There is no significant difference of dust exposure level between rice mill workers and management workers.

2. Alternative hypothesis:

   There is significant difference of dust exposure level between rice mill workers and management workers.

### *1.3.2 Hypothesis 2*

1. Null hypothesis:

   There is no significant difference of endotoxin exposure level between rice mill workers and management workers.

2. Alternative hypothesis:

   There is significant difference of endotoxin exposure level between rice mill workers and management workers.

*1.3.2 Hypothesis 3*

1. Null hypothesis:

   There is no significant difference of lung functions between rice mill workers and management workers

2. Alternative hypothesis:

   There is significant difference of lung functions between rice mill workers and management workers

## 1.4 Significance of the study

The significance of this study is to evaluate the endotoxin exposure level among rice mill workers who are at high risk of respiratory effects as they are in direct contact with rice dust. Moreover, it will encourage agricultural sector to develop farm machinery and agricultural technology which can reduce exposure to rice dust and lower the incidence of occupational diseases. This study also promote health awareness among workers involving knowledge, attitude and safety practices about personal protective equipment (PPE) and gives benefit for other researchers for further investigation about rice dust in Malaysia.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Rice mill

Rice (*Oryza sativa L.*) is the seed or kernel of paddy that consists of two different layers, inner layer (bran) or outer layer (husk) (Singha, 2013). The rice fruit is a caryopsis in which the single seed is fused with the wall of the ripened ovary (pericarp) that formed a seed-like grain whereas the grain is the ripened ovary, lemma, palea, rachilla, sterile lemmas and the awn (Figure 2.1). The lemma, palea, sterile lemmas, rachilla and the awn whenever present constitute the hull or husk (Bardenas & Chang, 1965).

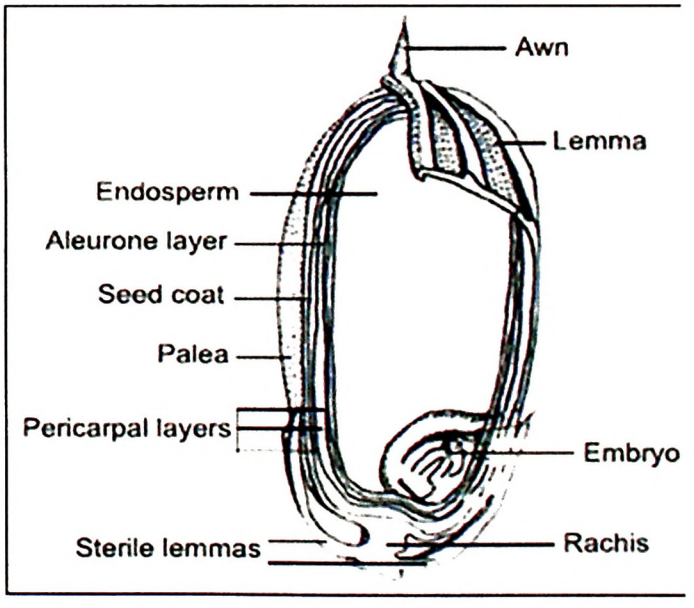


**Figure 2.1:** The structure of the rice grain (Adapted fromWopereis et al., 2009).

Maclean (2002) stated that the major rice producing countries are China, India, Indonesia, Bangladesh, Vietnam, Thailand, Myanmar, Philippines, Japan and Brazil. The production of rice in Asian region are approximately 90% of the total global rice output to which China and India contribute 28.7% and 19.5% shares of the total output, respectively (Esa et al., 2013). China is the largest producer and consumer of rice in the world and also a pioneer of hybrid rice technology which has increase China's grain output over the past 30 years (Figure 2.2) (Wang et al., 2005).

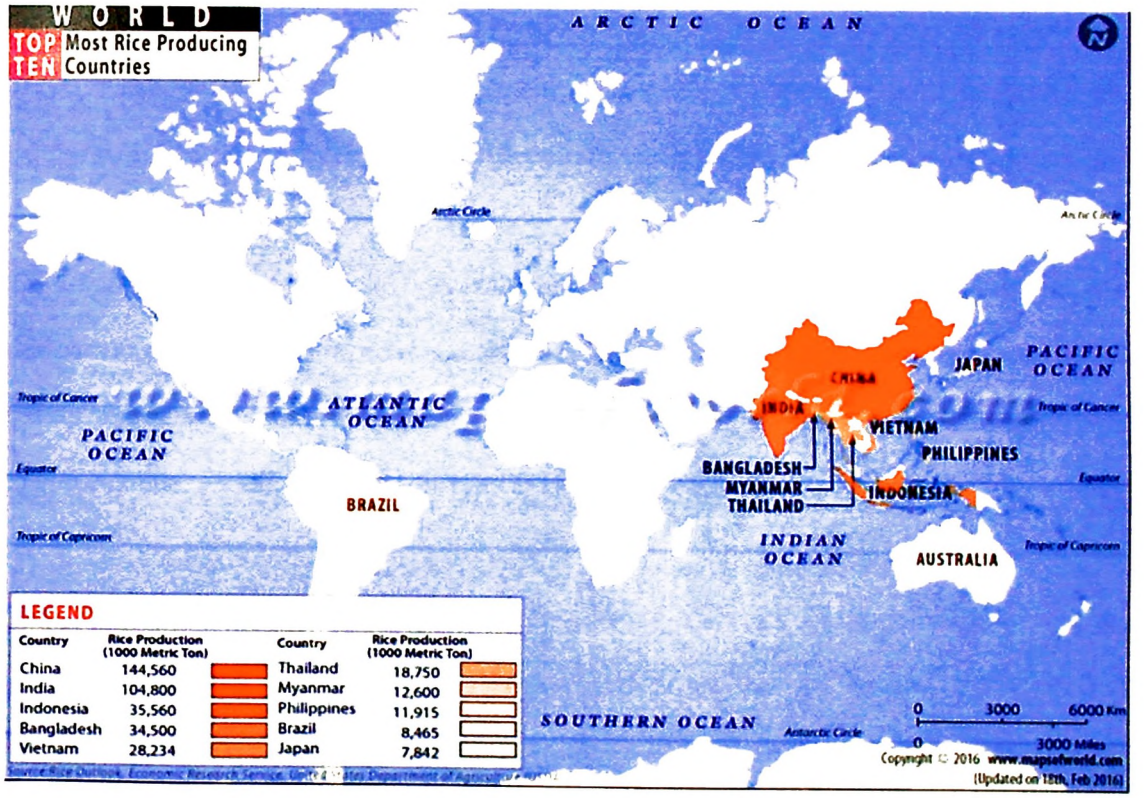


**Figure 2.2:** Top ten countries with the most rice producing countries (Adapted from map of the world, 2016).

Malaysia is a Southeast Asian country located partly on the Peninsula of the Asian mainland and partly on the northern third of the island of Borneo. Malaysia is a rain forest country. Previous study stated that Malaysia is not ideal for rice planting due to the potential impacts of climate change on the rice production (Vaghefi et al., 2011). Krishnan et al. (2007) stated that climatic variability and the predicted climatic changes are a major concern to the rice crop scientists because of the potential threat to the rice productivity. If the $CO_2$ concentration is increase from 383 to 574 ppm with 2°C rise in temperature, the rice yield will decrease by 0.69 t $ha^{-1}$ (Vaghefi et al., 2011).

However, the Malaysian government is very supportive in rice farming activity and in the irrigation development. About fifteen rice varieties have been breed to tolerate high temperature in Malaysia (Singh et al., 1996; Teik, 1985). Previous study stated that one of major strategies of Malaysian government was eight granary areas are designated as permanent paddy producing areas (Vaghefi et al., 2011). Nowadays, the production of rice paddy in Malaysia increased from 2036600 MT in 1999 to 2510000 MT in 2009 with average annual growth rate of 1.7% (FAO, 2011).

Kelantan is located at the northeast coast of Malaysia and encounters a very dry period from March to June and heavy rains from November to January or February. In Kelantan, rice production is one of the most important activities in agricultural or industry pursuits. Fujimoto (1976) discussed that the rice production level in Kelantan remains lower than the national average in the northeastern state despite Kelantan has the second largest land area devoted to rice planting in Malaysia.

Rice milling is a process to remove the husk and part of the bran from paddy to produce edible rice (Shrivastava & Soni, 2011). The rice mill process consists of pre-cleaning, dehusking or dehulling, paddy separation, whitening or polishing, grading, separation of white rice, mixing, mist polishing and weighing of rice (Figure 2.3).

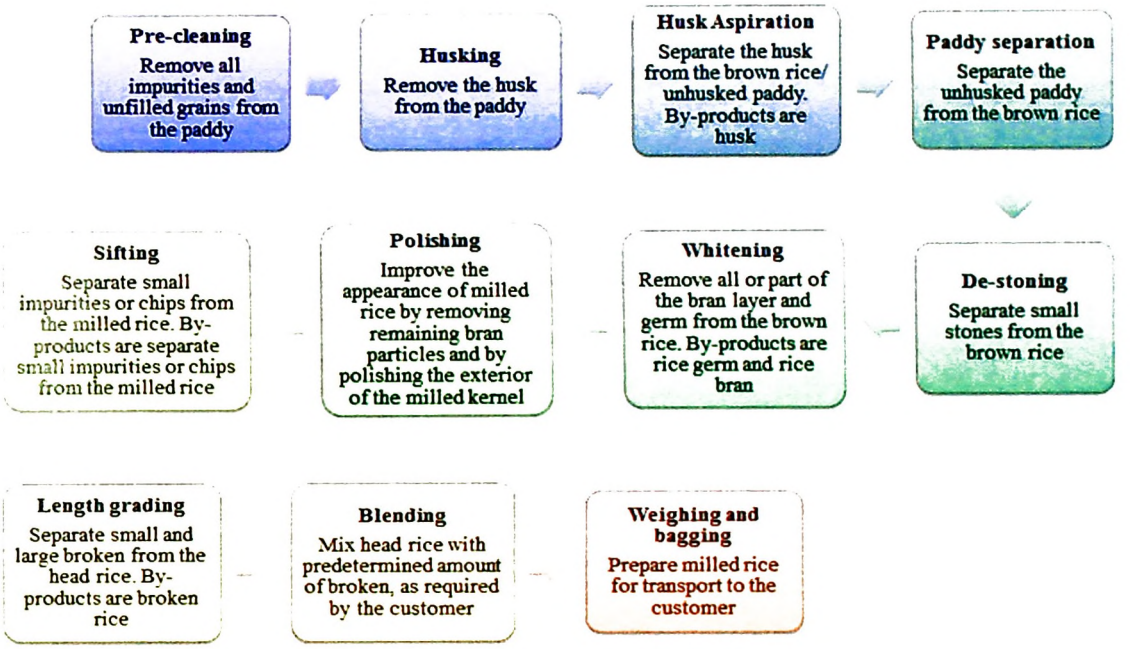


**Figure 2.3:** The rice milling process (Adapted from International Rice Research Institute (IRRI), 2009).

The primary milling operation involved in rice milling process is important to remove the husk and bran layers after harvesting and drying (Dhankhar, 2014). The quality and quantity of rice grains depend on the milling process. The impurities of white rice also depend on the milling process. For an ideal milling process, the fractions must be around 20% husk and 8% to 12% bran based on the milling degree and 68% to 72% milled rice or

white rice (IRRI, 2009). Improvement of the milling process gives good yield, good quality and higher net returns on investment (Singha, 2013).

### 2.2 Inhalable rice dust

According to the "Glossary of Atmospheric Chemistry Terms" (IUPAC, 1990) (as cited in World Health Organization (WHO), 1999), *"Dust is small, dry, solid particles projected into the air by natural forces such as wind, volcanic eruption and by mechanical or man-made processes such as crushing, grinding, milling, drilling, demolition, shoveling, conveying, screening, bagging and sweeping."* Dusts are defined as solid particles (1 μm – 100 μm) that become airborne depend on their origin, physical characteristics and ambient conditions (WHO, 1999). Figure 2.4 shows the different regions of the nasopharyngeal or extrathoracic system, tracheobronchial and alveolar.

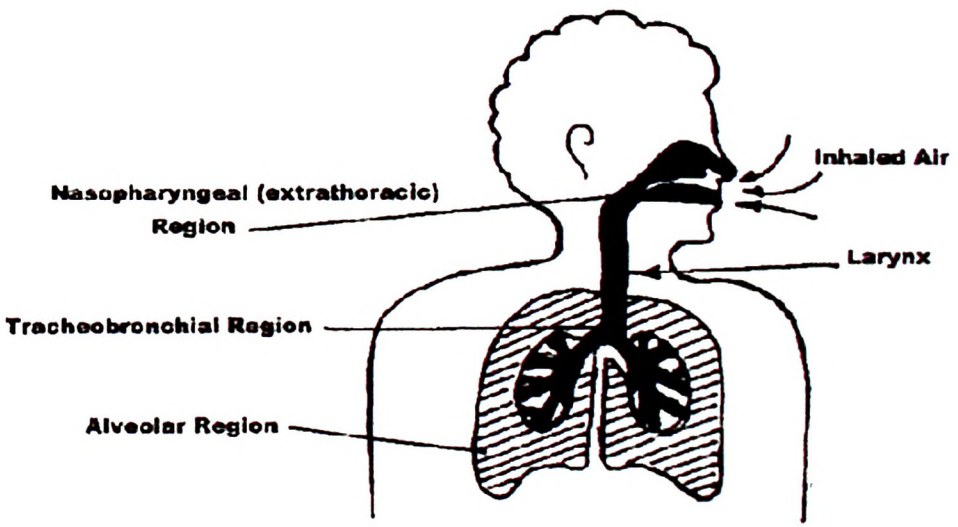


**Figure 2.4**: Anatomy of respiratory system (Adapted from WHO, 1999).

Respiratory tract are divided into three main regions; tracheobranchial region, alveolar region and nasopharyngeal region. In terms of a mass fraction, inhalable fraction is the mass fraction of total airborne particles entering the respiratory system via the nose or mouth, extrathoracic fraction is the mass fraction of inhaled particles fail to penetrate beyond the larynx, thoracic fraction is the mass fraction of inhaled particles penetrate beyond the larynx and respirable faction is the mass fraction of inhaled particles entering the non-ciliated alveoli (Norme, 1993).

WHO (1999) found that airborne particles are inhaled depending on its aerodynamic diameter, the velocity of the surrounding and the person's breathing rate. In coal miners, the small dust caused pneumoconiosis and silicosis whereas the larger size fraction of dust caused bronchitis and obstructive changes in pulmonary function (Morgan, 1978). Brown et al. (2013) studied that less than 20% penetration of 10 μm particles into extrathoracic airways. Penetration of particles into thoracic and respiratory region of children were greater than adults (Brown et al., 2013). Several study also stated that 10 μm particles (50%) penetrate into thoracic whereas 50% penetration of 4.0 μm particles into respiratory fractions (Norme, 1993; TLVs, 2004).

The small airborne particles entered the nose (nasal route) or mouth (oral route) during inhalation. WHO (1999) stated that airborne particles greater than 30 μm are deposited in the airways of the head, which is the air passages between the point of entry at the lips or nares and the larynx. If the particles failed to deposit in the head, it will enter the tracheobronchial airway region or be eliminated by mucociliary clearance or if soluble may

enter the body by dissolution. The smaller particles penetrated to the alveolar region and will be absorbed by the blood. Other study stated that all inhaled particles smaller than 10 μm in diameter can enter the respiratory tract and can potentially be biologically active in susceptible individuals (Heyder, 2004).

Inhalable dust or airborne dust is related with the field of environmental hygiene which is more concerned with pollution of the general atmospheric environment (WHO, 1999). Dust is presence in the rice during rice processing which is from cleaning, husking, milling, polishing, grading, sorting and storage. However, previous study found that increase in the concentration of large dust during harvesting contributed to the increase in the concentration of the fungal spores (Lee et al., 2006). Rice dust can also be contaminated with other factors such as microorganism, plants, animals, feather, pollens, silica, soil particles, fungicides, pesticides or hair (Schenker et al., 1999).

The rice mill workers are highly exposed to inhalable rice dust that contain rice husk which can cause various adverse effects to human body. Rice husk may contain microorganism, spores, chemicals, pesticides, viable particles and organic dust that can be inhaled through the nose or mouth and enter the lung (WHO, 1999). Lack of health awareness and safety are the factors that contribute to adverse health risks (Musa, 2006). Rice dust can cause major adverse effect on respiratory system and lung (Musa et al., 2000; Ye et al., 1998). For many years, its effects on various organs had been reported such as inflammatory disease of the eyes, nose and skin which may be caused by direct irritation and immune-based reactions (Hurst & Dosman, 1990).

According to Tripathi et al. (2014), rice husk dust can induce kerato-conjunctival irritation, corneal scars, chronic conjunctival inflammation, pterygium formation, allergic inflammatory reaction and pruritus. Lim et al. (1984) discussed that rice dust exposure can cause acute and chronic irritant effects of eyes, skin, upper respiratory tract; allergic responses such as nasal catarrh, tightness of chest, asthma, eosinophilia; radiological opacities in the chest, early silicosis or extrinsic allergenic alveolitis known as "rice millers' syndrome". However, there are several factors that can contribute to occupational diseases among rice mill workers such as constitution and dust concentration, duration of exposure, variation in personal immunity, different activity and seasons (Dewangan & Patil, 2015).

**2.3 Endotoxins**

Endotoxin which is also known as lipopolysaccharide (LPS) comprises of an O-specific chain, a core oligosaccharide and a lipid component, termed lipid A (Rietschel et al., 1994). Endotoxins are a part of outer membrane of gram-negative bacteria that can induce potent pathophysiological effects (Figure 2.5).

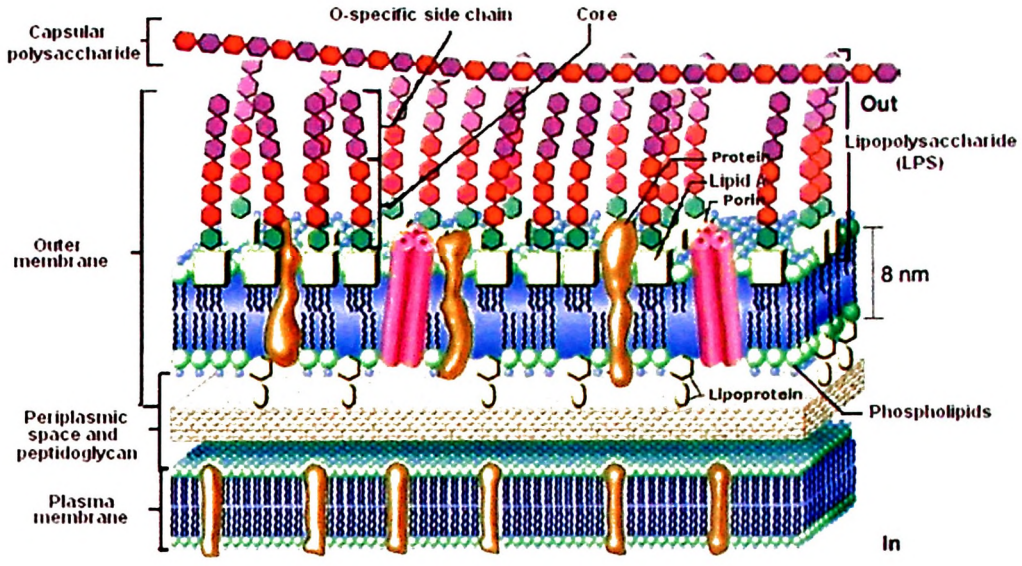


**Figure 2.5:** Gram-negative bacteria cell membrane (Adapted from Karunaratne, 2012).

LPS is a potent activator of macrophage functions that produces various cytokines such as tumor necrosis factor alpha (TNF-α), IL-1, interleukin-6 (IL-6), interleukin-10 (IL-10) and macrophage inflammatory protein-1α/β inflammatory effector substance such as protanoids, leukotrienes and nitric oxide (NO) enhance expression of cell surface antigens such as major histocompatibility complex (MHC) class II and B7-1/2 (Ulevitch, 1993). Workers who are exposed to endotoxin can experience several health risks effects. Figure 2.6 shows the effects after endotoxin released in the body.

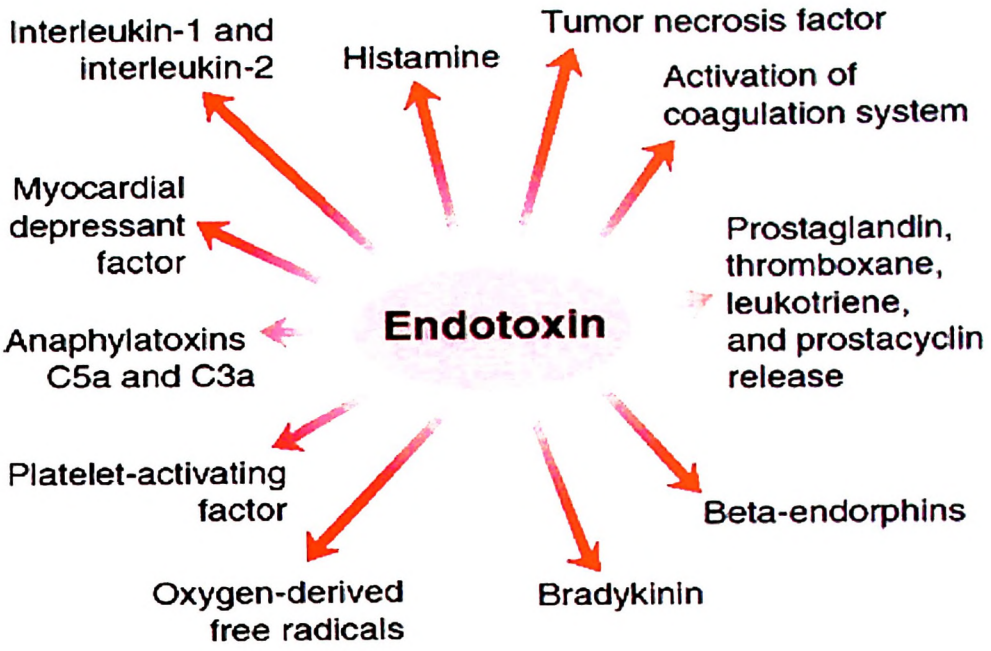


**Figure 2.6**: Effects of endotoxin release (Adapted from Miller & Keane, 2003).

Several lines of evidence indicated that endotoxin is a part of the primary agents in dust related to the acute changes in airway physiology and airway inflammation. Martinez and Holt (1999) explained that endotoxins are potent stimulators of the immune system. Low level of endotoxin exposure can minimise the risk of developing atopic disease in childhood (Von Mutius et al., 2000). Endotoxin release large amount of TNF and IL-1 that can cause tissue injury and lethal shock (Wang et al., 1999). Organic dust such as animal faeces, plant materials and bacteria contaminated can cause endotoxin exposure (Spaan et al., 2006). Previous studies had demonstrated that people who work in healthy offices buildings exposed to large concentration of dust bacteria shown high risks of work-related disease (Bouillard et al., 2005).

Silverman and Ostro (1999) stated that endotoxin can be transmitted into the blood that cause endotoxemia in two ways whether through local or systemic infection by exogenous gram-negative bacteria or translocation of endogenous gram-negative bacteria or fragments across the intestinal membrane when permeability is increased in systemic insults such as trauma. The presence of endotoxemia induce inflammatory response; activate various interrelated non-cellular cascades during LPS binding to lipopolysaccharide binding protein (LBP), the LBP-LPS complex by stimulating from the immune system and sepsis (Silverman & Ostro, 1999). Sepsis can lead to manifest disorientation, end organ dysfunction, somnolence, asterixis and portasystemic encephalopathy (Jeppsson et al., 1981; Rangel-Frausto, 1999).

The patients with septic shock have higher mortality risk compared with critically ill patients who were nonseptic (Annane et al., 2003). The researchers concluded that the symptoms of septic shock are the result of the actions of host cytokines induced by the endotoxin, IL-1 as a mediator of septic shock that can induce tachycardia and hypotension and act synergistically with tumour necrosis factor that lead to tissue damage and death. Fischer et al. (1992) discovered that IL-1 of *Escherichia coli* bacteria contributes to the hemodynamic and metabolic consequences of sublethal endotoxemia or gram-negative septic shock in non-human primates.

TNF-α is involved in the regulation of immunity and inflammation (Kock et al., 1990). TNF-α produces myocardial depression, dilation and cardiovascular patterns of spontaneous septic shock which decreased arterial pressure, systemic vascular resistance

and ejection fraction among canine and human (Cunnion & Parrillo, 1989). Other than that, TNF-α is capable to induce NO synthase (NOS) in glia that relate with cardiovascular disease (Forstermann & Munzel, 2006; Romero et al., 1996). Moreover, increased in TNF-α gene expression in the hepatic tissue plays a role for endotoxaemia in the development of steatohepatitis in obese patients due to elevated plasma levels of LPS-binding protein (LBP) (Ruiz et al., 2007).

Endotoxin is also related with a myriad of disease and syndrome including complications associated with sepsis, liver failure (e.g. cirrhosis), burns, inflammatory bowel diseases, pancreatitis, trauma, invasive surgery, as wells as organ-specific illnesses such as cystic fibrosis, kidney dialysis complications, asthma and autoimmune diseases (Dick, 2012; Foteinou et al., 2007; Manthous et al., 1993). Myriad of physiologic parameters act as prognostic markers in sepsis and septic shock include blood pressure, vascular resistance, cardiac index, other derived hemodynamic parameters, systemic oxygen delivery, tissue oxygen consumption, anion gap and gastric tonometry (Rhodes) (Silverman & Ostro, 1999).

**2.4 Lung function**

The major function of lung is to help oxygen entering the circulatory system during inhalation and remove carbon dioxide during exhalation. Inhaled oxygen entered the lungs and reached the alveoli. On the surface of the alveoli, a distance of approximately 1 mm – the movement of gases is by diffusion (Cotes et al., 2006). Oxygen is diffused across the alveolar wall into plasma in the alveolar capillary and chemical combination with

haemoglobin present in the red blood cells (Cotes et al., 2006). Based on Figure 2.7, the lungs are a pair of spongy, air-filled organs located in the chest.

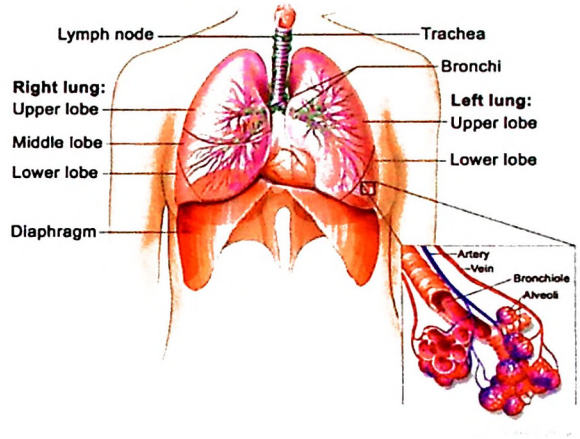


**Figure 2.7:** The respiratory system (Adapted from National Cancer Institute, 2016).

Lung failure can occur when fluid builds up in the air sacs and lung cannot release oxygen into blood or remove carbon dioxide from blood. Then, the organs cannot get enough oxygen-rich blood to function that cause acute respiratory failure. Lung failure is commonly associated with dust exposure in rice mill or other industry related with airborne transmission. Weill et al. (1975) discovered that increased cumulative dust exposure in rice mill workers cause decreased of lung volumes and maximum expiratory flow rates. Noninfectious, febrile illness associated with chills, malaise, myalgia, a dry cough, dyspnea, headache and nausea due to heavy organic dust exposure or also known as organic

dust toxic syndrome shown acute lung disease, hypersensitivity pneumonitis and also increase numbers of neutrophils in bronchoalveolar lavage (Von Essen et al., 1990).

**2.5 Spirometer**

Spirometry is usually used for lung function test (LFT) to measure the movement of air from the lungs during various breathing maneuvers. Spirometry is used for patient with respiratory symptoms such as chronic cough, episodic wheezing and dyspnea disorder to detect airways obstruction due to asthma or chronic obstructive pulmonary disease (COPD) (Ferguson et al., 2000). LFT is used to diagnosis obstructive abnormalities, restrictive abnormalities, bronchiodilator response, central and upper airway obstruction, or mixed ventilator defect (Pellegrino et al., 2005). LFT is useful to identify abnormalities of lung function that might otherwise be overlooked including the possibility of some respiratory disorders such as COPD (Crapo, 1994).

The parameters used to measure lung function are forced expiratory volume in one second (FEV1), forced vital capacity (FVC) and FEV1/FVC%. Reduced of FEV1 may reflect restrictive lung function, lung cancer, coronary artery disease (CAD), stroke, COPD, obesity, type 2 diabetes, heart failure and also remain a marker of overall mortality (Davis et al., 2000; Gelb et al., 1983; Mannino et al., 2005; Young et al., 2007). FVC is a reliable, valid and responsive measure of pulmonary function in idiopathic pulmonary fibrosis (du Bois et al., 2011). FEV1/FVC% or FEV1/vital capacity (VC) is considered as "de facto gold standard" for detection of obstructive ventilator defect by a reduced below the $5^{th}$ percentile of the predicted value (Pellegrino et al., 2005; Vandevoorde et al., 2008).

# CHAPTER 3

# MATERIALS AND METHODS

## 3.1 Study location

Samples were taken among exposed group at Kilang Padi Liangtong (K.B.) Sdn. Bhd. located in Kota Bharu, Kelantan. Dust samples of the non-exposed were collected at Kilang Padi Liangtong (K.B.) Sdn. Bhd. and Universiti Sains Malaysia (USM) located in Kubang Kerian, Kelantan.

## 3.2 Study participants

Samples were collected from permanent rice mill workers (n=10), USM management workers (n=5) and rice mill management workers (n=5). Inclusion criteria are permanent rice mill workers, subjects have been exposed to rice dust particles and management workers who are not exposed to rice dust particles. Exclusion criteria for subjects are temporary or part-time or contract workers and management workers. Ethical approval (USM/JEPeM/1405196) was granted by the Human Research Ethics Committee, USM (Appendix B). Consent form and letter of consent were also attached in Appendix A and C.

## 3.3 Materials

### 3.3.1 Chemicals preparation

a. 70% Ethanol

70 ml of 100% ethanol were dissolved in 30 ml of distilled water. 70% of ethanol was used for sterilising surfaces or aseptic technique in a lab setting. 70% ethanol was stored at 4 °C.

b. 0.1% phosphate buffer saline with Tween-20 (PBST)

1x PBS was made by adding 10 tables of PBS in 1 000 mL of distilled water. Then, 1 mL of Tween-20 added in 1 000 mL of 1x PBS to make 0.1% (v/v) Tween-20 in 1x PBS. 0.1% PBST was used to preserve dust sample before extraction. 0.1% PBST was stored at 4 °C.

c. 0.1 N Sodium hydroxide

4.0 g of sodium hydroxide added in distilled water to make 1 000 mL.

d. 0.1 N Hydrochloric acid

9.9 mL of 37% Hydrochloric acid and 990.1 mL of distilled water mixed to get 1000 mL.

e. Limulus Amebocyte Lysate (LAL)

1.7 mL LAL reagent water was added to reconstitute lyophilised lysate. Reagent was swirled gently for 30 seconds and stored at -20 °C for one week.

f. Chromogenic substrate

1.7 mL LAL reagent water was added to reconstitute lyophilised lysate. Reagent was stored at 4 °C for one month.

g. Stop solution

10 mL of buffer S was added to reconstitute the color-stabiliser #1 and stored at 4 °C for one week.

h. Color-stabiliser #2 and #3

10 mL of LAL water was added into each color-stabiliser and stored at 4 °C for one week.

i. Standard endotoxin solution

2 mL of LAL reagent water was added to dissolve lyophilised endotoxin standard and mixed with vortex shaker for 15 minutes. Reagent was stored at 4 °C for one week. Then, serial endotoxin standard solution was made.

### 3.3.2 Reagents for LAL test (GenScript ToxinSensor™ Endotoxin Detection System, USA).

LAL reagents water, *Limulus Amebocytes Lysate* (LAL), *E. coli* endotoxin standard, chromogenic substance, buffer S for color-stabiliser #1, color-stabiliser #1, color-stabiliser #2, color-stabiliser #3.

### 3.4 Laboratory equipments and consumables

Biosafety cabinet, pipette (20-200 µL and 200-1000 µL), pipette tips, 40 mL urine container, glass-fiber filters (1.0 µm pore, 25 mm diameter), powder-free gloves, IOM sampler, personal air sampling pump (Gilian GilAir Plus, US), 15 mL centrifuge tubes, 1.5 ml microcentrifuge tubes, forceps, tissue roll, vortex mixer, orbital shaker (Heidolph Rotamax 120 shaker, Germany), centrifuge machine (Hettich Universal 32R, Germany), water bath, timer, test tube rack, spectrometer (Thermo Scientific GENESYS 20, USA), spirometer (Cosmed Pony FX desktop, Italy) and paper mouthpieces.

### 3.5 Methods

#### 3.5.1 Airborne sampling

The procedure of samples collection followed the standard operating procedure (SOP) for determination of hazardous substances (MDHS 14/4) (HSE, 2000).

*3.5.1.1 Pre-weight of filter*

Powder-free gloves were worn. The IOM sampler was disassembled. The External surface of unloaded IOM cassette was wiped with an alcohol swab. The control filter (0.1 µm, 25mm GFA) and sample filter (0.1 µm, 25mm GFA) with cassettes and protective cap were placed on the analytical balance and were weighted. This was recorded as pre-weight. The control filter was removed from IOM sampler and placed in urine container.

*3.5.1.2 Sampling*

The sample filter was inserted in IOM sampler. The worker's clothing was clip in the breathing zone. The protective cap was removed and the pump was switch on. The time was recorded. Sampling was carried out for 8 hours in which the workers were free to continue with their work as usual. After sampling, the pump was stop. The time was recorded and the protective cap was reattached. The filter was removed from the IOM sampler and placed into a urine container. The external surface of the cassette was wiped with an alcohol swab.

*3.5.1.3 Post-weight filter*

Post-weight filter with dust was recorded to measure the weight of the samples collected.

### 3.5.2 Sample storage and transportation

After the filter has been weighed, the filter sample was placed in a test tube containing 1 ml of 0.1% PBST and stored at -20 °C until analysis.

### 3.5.3 Extraction of dust samples

Before the extraction of dust samples, the samples were thawed. In the biosafety cabinet, the samples were mixed with PBST by using vortex-genie mixer for 1 minute. Then, the samples were shaken for an hour at room temperature on an orbital shaker. The samples were centrifuged 1331 G-force for 10 minutes. Finally,