EFFECTS OF ENDOTOXIN EXPOSURE ON RESPIRATORY HEALTH AMONG RICE MILL WORKERS; FROM WORKPLACES TO HOMES

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by

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LIST OF ACRONYMS, ABBREVIATIONS AND SYMBOLS

%	Percent
µg	Microgram
µl	Microliter
ARTP	Association for Respiratory Technology and Physiology
B	Beta
BERNAS	Beras National Berhad
BLL	Blood lead level
BMRC	British Medical Research Council
BSL	Biosafety level
BS EN	British Standard European Norm
CD 14	Cluster of differentiation
CI	Confidence Interval
cm	Centimeter
COPD	Chronic obstructive pulmonary disease
CRP	C-reactive protein
DECOS	Dutch Expert Committee on Occupational Exposure
df	Degree of freedom
DOSH	Department of Safety and Health
ECP	Eosinophilic cationic protein
Et al	*Et Alta (and others)*
EU/m^3	Endotoxin units per cubic meter
EU/mg	Endotoxin unit per miligram
EU/ml	Endotoxin Unit per mililiter

f/cc	Fiber per cubic centimeter
FEF	Forced expiratory flow
$FEF_{0.2-1.2}$	Forced expiratory flow at 0.2-1.2% of forced vital capacity
$FEF_{25-75\%}$	Forced expiratory flow between 25% and 75% of FVC
$FEV_{0.5}$	Forced Expiratory Volume in 0.5 seconds
FEV_1	Forced expiratory volume in the first second
FEV_3	Forced expiratory volume in the three seconds
FEV_1/FVC	Ratio of forced expiratory volume in the first second to forced vital capacity
FMA	Factory and Machinery Act
FVC	Forced vital capacity
GFA	Glass fiber filter
H_1	Alternative Hypothesis
H_o	Null Hypothesis
IOM	Institute of Occupational Medicine
IQR	Interquartile range
ISO	International Organisation for Standardisation
JePEM	Jawatankuasa Etika Penyelidikan Manusia
K. A. P	Knowledge, attitude and practices
KB	Kilang Beras
KBB	Kilang Beras BERNAS
kg	Kilogram
L/min	Liter per minute
L/s	Liter per second

LAL	Limulus Amoebocyte Lysate
LFT	Lung function test
LOD	Limit of detection
LPS	Lipopolysaccharide
m2	Meter square
MD 2	Lymphocyte antigen 96
mg/m3	Milligram per meter cube
ml	mililiter
mm	Milimeter
MMI	Mucosal membrane irritation
MPO	Myeloperoxidase
MyGAP	Malaysian Agriculture Practice
NEHAP	National Environmental Action Plan
NICE	National Institute for Health and Care Excellence
NIOSH	National Institute for Occupational, Safety and Health
ºC	Celcius
OLD	Occupational lung disease
OR	Odd ratio
PBS	Phosphate buffer saline
PBST	Phosphate buffer saline with Tween 20
PEFR	Peak expiratory flow rate
PEL	Permissible exposure limit
PM	Particulate Matter
PM2.5	Particulate matter that have diameter less than 2.5

	micrometers
PPE	Personal Protective Equipment
R2	Coefficient of Determination
ROC	Receiver operating caharacteristics
SD	Standard deviation
SOP	Standard Operating Procedure
SPSS	Statistical Packages for Social Sciences
t	Time
TLR-4	Toll-like receptor
USM	Universiti Sains Malaysia
Vs	Volume of air sampled
WHO	World Health Organisation
ΔFEV1	Change in FEV1 compared to baseline FEV1
ΔFVC	Change in FVC compared to baseline FVC
χ2	Chi-square

KESAN PENDEDAHAN ENDOTOKSIN TERHADAP KESIHATAN RESPIRATORI DALAM KALANGAN PEKERJA KILANG BERAS; DARI TEMPAT KERJA KE RUMAH

ABSTRAK

Kajian pendedahan pekerjaan terhadap endotoksin telah meluas dan tahap kepekatan endoksin yang dikesan adalah tinggi meliputi pelbagai sektor dan industry. Pendedahan telah memberi kesan kesihatan seperti gangguan pernafasan, asma, bronkitis yang kronik, dan penyakit pulmonari obstruktif kronik. Jalan utama pendedahan adalah melalui penyedutan. Malah, pencemaran endotoxin tersebut mungkin dipindahkan kepada ahli keluarga perkerja terdedah. Oleh itu, kajian keratan rentas perbandingan ini bertujuan untuk mengkaji penyebaran endotoksin dalam habuk beras dari tempat kerja ke rumah dan kesannya terhadap pernafasan dalam kalangan pengilang beras di Malaysia dan kakitangan pentadbiran Kampus Kesihatan USM. Tahap endotoksin dalam habuk tersedut bagi penyempelan kawasan dan peribadi dilakukan menggunakan penapis Fiber Glass (A) berdiameter 25 mm yang dimuatkan ke dalam penyampel IOM yang disambungkan pada pam dan diklipkan pada kolar pekerja selama lapan jam dan. Pembawaan debu beras ke rumah dikaji dengan mengambil sampel sapuan tangan selepas waktu bekerja dan sampel vakum dirumah daripada kedua-dua kumpulan. Soal selidik diedarkan dalam kalangan peserta yang terdiri daripada enam bahagian; maklumat peribadi, maklumat pekerjaan, PPE, amalan keselamatan di tempat kerja, gejala pernafasan dan pendedahan di rumah. Sampel disimpan pada suhu -20ºC dan dianalisis dengan menggunakan *Limulus Amoebocyte Lysate Chromogenic Endpoint assay* pada 405 nm. Ujian fungsi paru- paru (LFT) telah dilakukan menggunakan Spirometer.

Hasilnya dianalisis secara statistik menggunakan SPSS Versi 24. Penemuan median kepekatan endotoksin untuk sampel kawasan adalah 0.32 EU/m^3 pada julat kurtil (IQR) 0.13 hingga 0.37 EU/m^3 manakala median untuk tahap endotoksin penyempelan peribadi dalam kalangan pengilang beras adalah 0.33EU/m^3 dengan IQR (0.14-0.34) EU/m^3. Terdapat penurunan yang tidak signifikan untuk sapuan tangan selepas kerja antara pengilang beras dan kakitangan pentadbiran dengan median (IQR); 0.06 (0.00-0.11) EU/ml berbanding 0.04 (0.02-8.37) EU/ml. Kebanyakan pengilang beras mengadu mempunyai batuk (32.4%, n = 24), kahak (27%, n = 20) diikuti oleh dyspnoea (16.2%, n = 12). Ujian fungsi paru- paru selepas kerja untuk FEV1/FVC yang diukur kelihatan lebih rendah dalam kalangan pengilang beras (58.36 ± 21.41) berbanding kakitangan pentadbiran (63.65 ± 19.26), tetapi secara statistiknya tidak signifikan ($p = 0.158$). Walau bagaimanapun, terdapat hubungan yang ketara antara kepekatan endotoksin dan parameter LFT selepas kerja yang diukur bagi FVC, FEV1, FEV1/FVC dan PEFR ($p <0.05$). Terdapat perbezaan yang signifikan dalam tahap endotoksin dalam habuk di rumah antara pengilang beras dan kakitangan pentadbiran, tetapi tahap endotoksin yang dapat dikesan adalah lebih tinggi dalam kalangan kakitangan pentadbiran berbanding pengilang beras dengan masing-masing median (IQR): 4.76 (0.1-6.35) EU/ml dan 0.21 (0.07- 0.21) EU/ml. Kajian semasa ini membuktikan bahawa tahap endotoksin yang dapat dikesan dalam kalangan pengilang beras di Malaysia adalah sangat rendah yang menunjukkan tahap selamat. Walaupun tahap pendedahan endotoxin adalah rendah, langkah perlindungan yang betul harus diterapkan dalam kalangan pekerja kilang beras bagi perlindungan jangka panjang.

EFFECTS OF ENDOTOXIN EXPOSURE ON RESPIRATORY HEALTH AMONG RICE MILL WORKERS; FROM WORKPLACES TO HOMES

ABSTRACT

The study of occupational exposure to endotoxin has been broad and high concentration of endotoxins detected across various sectors and industries. These exposure leads to the health effects which include respiratory disorder, asthma, chronic bronchitis and chronic obstructive pulmonary disease. The major route of exposure is through inhalation. In fact, the contamination might be transferred to the family members of the exposed workers. Thus, this comparative cross-sectional study aims to study the workplace to home transmission of endotoxin among rice millers in Malaysia and administrative staffs of USM Health Campus and their respiratory effects. Endotoxin level in inhalable dust for both area and personal samplings were collected using 25 mm Glass Fiber (A) filter loaded in IOM sampler connected to a pump which was clipped at the collar of the rice millers for eight hours. Take-home exposure route was studied by taking the post-shift hands wipes and vacuum home dust from both groups. The questionnaires were circulated among the participants where it consists of six parts; personal details, occupational details, PPE, safety practices at work, respiratory symptoms and take-home exposure. The samples were stored at -20ºC and analysed using Limulus Amoebocyte Lysate (LAL) Chromogenic Endpoint assay at 405 nm. The lung function tests (LFT) were carried out using Spirometer. The results were statistically analysed using SPSS Version 24. Findings for the median concentration of endotoxin for areas and personal inhalable were 0.32 EU/m^3 with Interquartile Range (IQR) 0.13 to 0.37 EU/m^3 and 0.33EU/m^3

with IQR (0.14-0.34) EU/m^3 respectively. There was no significant decline in post-shifts hand wipes between rice millers and administrative staffs each with the median (IQR); 0.06 (0.00-0.11) EU/ml and 0.04 (0.02-8.37) EU/ml. The administrative staffs have higher detectable of endotoxin in the vacuum home dusts, compared to rice millers with median (IQR); 4.76 (0.16-6.35) EU/ml and 0.21 (0.07-0.21) EU/ml respectively. Most of the rice millers complained having cough (32.4%, n=24), phlegm (27%, n=20) and dyspnoea (16.2%, n=12). Post-shift LFT for FEV1/FVC measured was lower among the rice millers (58.36 ± 21.41) % compared to the administrative staffs (63.65 ± 19.26) %, but was not statistically significant ($p = 0.158$). However, there were significant correlations between endotoxin concentration and post-shift LFT parameters of measured FVC, FEV1, FEV1/FVC and PEFR ($p < 0.05$). There was significant difference in home dust endotoxin level between these two groups, but higher detectable endotoxin level found among administrative staffs compared to the rice millers with median (IQR): 4.76 (0.1-6.35) EU/ml and 0.21 (0.07-0.21) EU/ml respectively. This current study has established that the endotoxin level detectable among rice millers in Malaysia was very low which indicating safe level. Despite low level of endotoxin exposure, yet proper protective measure should be applied among the rice millers for long term protection.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Exposure to endotoxin leads to both acute and chronic effects. The acute effects involve various kinds of respiratory symptoms such as cough, dyspnoea, asthma, breathlessness, chest tightness, shivering and joint aches. Meanwhile, chronic effects may lead to chronic bronchitis, decline in lung function and developing of chronic obstructive pulmonary disease (COPD) (Senior *et al*., 2015).

The sources of exposure of endotoxins are varied from composting facilities, intensive farming, spreading of biosolids to land to the waste treatment both in rural areas as well as the urban areas (Rolph *et al*., 2018). The study of endotoxins exposure at the various workplaces such as cotton mills, rice mills, composting plant had shown high detectable of endotoxins (Ghani *et al*., 2016; Hendrarinata *et al*., 2015; Deacon *et al*., 2009).

The mostly reported mechanism of exposure is by inhaling the airborne endotoxin at the workplaces (Spaan *et al*., 2006) which in turn triggers the inflammation in respiratory systems. These airborne endotoxins originate from organic products contaminated with bacteria. Under specific condition, microbiological growth occurs during culturing, processing, storage and transport of agricultural products. Those activities lead to endotoxin to be airborne and inhaled by the exposed workers.

Apart from that, workplace exposure can also be a concern among workers' family members as the workers might 'take-home' the dust from work to their homes exposing their family members. Clothes, skin and vehicles which are contaminated with the endotoxin might be a ‘vehicle’ that bring these gram-negative bacteria to homes.

In Malaysia, apart from palm oil industries, rice processing industry is among the leading agricultural industry since rice is the staple food of the country. The consumption of rice in the year 2016 was about 2.7 million metric tonnes where 67% was produced locally and another 33% were imported from Thailand, Vietnam and Pakistan. Nearly about 200,000 of farmers and rice millers involve in this production of rice (Omar *et al*., 2019) exposing these workers to the contamination of bacterial endotoxin.

Previous study among rice millers in Kelantan found these workers complains various respiratory symptoms for example chest tightness and morning phlegm (Musa *et al*., 2000). Although early investigation showed the sources of the symptoms were rice husk dust, a lot of studies from various country linked those symptoms with the presence of bacterial endotoxins.

Malaysia government through the Ministry of Agriculture and Agro-Based Industry, had realised this situation and planned various actions to encounter this problem at the source. The organisation had introduced Farm Accreditation Scheme which accredits farms that adopt Malaysian Agriculture Practice (MyGAP) to ensure good practice of planting were implement therefore high quality of product are produced along with both environment and workers are protected (Jabatan Pertanian Malaysia, 2013). Apart from that, the Department of Occupational Health and Safety (DOSH) under Ministry of Human Resources has its role in ensuring the occupational safety and health at the workplace showing Malaysia emphasising the importance of safety and health practices at the workplace.

Exposure to bacterial endotoxin is still new in Malaysia even though a few studies has been conducted among office workers (Lim *et al*., 2019) and among school pupils in Johor Bahru (Norbäck *et al*., 2014). The data was still insufficient for the government to regulate new legislation as the government needs to collect information on both exposures and the health effects at the workplaces in order to understand specific occupational health problem (Rampal and Mohd Nizam, 2006). Since there is no enactment on the permissible exposure to endotoxin by Factory and Machinery Act of Malaysia (FMA) as well, therefore, exposure limits of endotoxins were referred to the Dutch Expert Committee on Occupational Safety (DECOS) in Netherland. DECOS' health-based recommended exposure limit is 90 EU/m^3 (eight-hour time-weighted average). This current study hopes to provide information on the exposure of endotoxin among rice millers in Malaysia in addition to possible pathway transmitting the contamination to the family members.

1.2 Problem Statement

There are about 400 rice mills commercially operated in Malaysia (Omar *et al*., 2019). In the year 2015, about 400,906 MT of paddy milled by BERNAS. This large amount of production exposing the rice millers hence increasing risk of workplace exposure to endotoxin. A statistic report released by the Malaysian Department of Occupational and Health showing an increasing number of occupational diseases and poisoning cases from 2005 to 2016. This trend however decreases by the year 2017 about 23% from 2016 before increasing again to 17% in the year 2018. Up until February 2019, 1785 cases were reported regarding occupational diseases. Of all the reported cases, occupational lung diseases (OLD) accounted about 0.95% and disease caused by the biological agent accounted about

0.68% (Occupational Health Division, 2019). Although it is showing a small number of cases, it should not be taken lightly.

Workers involved in the production of rice were prone to have various types of airways diseases such as pneumoconiosis, Farmer's Lung, chronic bronchitis, pulmonary fibrosis and asthma. The needle like structure of rice dust husk causes chronic irritation of bronchial which in turn result in the impairment of lung function. It also brings damages to the bronchial passage as well as the elasticity of the alveolar walls (Kaur., 2011).

These irritants affecting the eyes, skin and upper respiratory tract; as well as allergic responses such as nasal catarrh, tightness of chest, asthma and eosinophilia; and radiological opacities in the chest, probably representing early silicosis or extrinsic allergic alveolitis. Sixteen years after that, Musa *et al.*, (2000) studied on lung function decline among rice millers in Kelantan, reported on chest tightness and shortness of breath as most common symptoms complained by the workers. Nevertheless, the agent that cause the respiratory symptoms as well as decline in lung function still remained unclear. Both previous studies in Malaysia do not investigate on the potential of endotoxin contained in the rice dust which might trigger the respiratory effects among rice millers (Lim *et al.*, 1984; Musa *et al.*, 2000).

However, a study conducted in the neighbouring country of Malaysia such as Indonesia had reported a significant correlation between presence of endotoxin in rice dust and its association with the decline in lung function among rice millers (Dimjati Lusno et al., 2018). Another study in Thailand also found restrictive lung function experienced by the rice millers (Batsungneon and Kulworawanichpong, 2011). The respiratory condition such as lung function decline among Malaysian, Indonesia and Thai rice millers have been well documented. However, the exposure assessments involving inhalational and dermal route linking parameters such as endotoxin in rice dust with the respiratory effect is scarce. In addition to that, the possible take-home pathway of endotoxin contaminating skin and clothing of rice millers is still not well discovered.

1.3 Research Aims, Objectives and Hypotheses

1.3.1 General Objectives

To study the workplace to home transmission of endotoxin in rice dust and its respiratory effects among rice millers.

1.3.2 Specific Objectives

i. To determine the concentration of endotoxin in airborne filter, post-wipes and home vacuum among rice millers and compared with administrative staffs.

ii. To determine the respiratory symptoms and lung function between rice millers and administrative staffs.

iii. To compare the lung function between rice millers and administrative staffs.

iv. To correlate the concentration of endotoxin in airborne filter, post-wipes and home vacuum with respiratory effects among rice millers.

v. To relate the levels of endotoxin exposure in airborne filter, post-shift wipes and vacuum home dusts with lung function among rice millers.

1.3.3 Hypotheses

Hypothesis 1

Null Hypothesis, H_O: There is no significant difference in concentration of endotoxin in post-shift hands wipe between rice millers and administrative staffs.

Alternative Hypothesis, H_1: There is significant difference in concentration of endotoxin post-shift wipes between rice millers and administrative staffs.

Hypothesis 2

Null Hypothesis, H_O: There is no significant difference in concentration of endotoxin in post-shift hands wipes and vacuum home dusts among rice millers compared to administrative staffs.

Alternative Hypothesis, H_1: There is significant difference in concentration of endotoxin in post-shift hands wipes and vacuum home dusts among rice millers compared to administrative staffs.

Hypothesis 3

Null Hypothesis, H_O: There is no significant difference in respiratory symptoms and lung function among rice millers compared to administrative staffs.

Alternative Hypothesis, H_1: There is significant difference in respiratory symptoms and lung function rice millers compared to administrative staffs.

Hypothesis 4

Null Hypothesis, H_O: There is no correlation between workplace, airborne, post-shift hands wipes and vacuum home dusts concentration of endotoxin among rice millers.

Alternative Hypothesis, H_1: There is correlation between workplace, airborne, post-shifts hands wipes and vacuum home dusts concentration of endotoxin among rice millers.

1.4 Conceptual Framework

In this research, rice mills are thought to be the main source of endotoxin exposure. The contaminants that released into the working environment and get exposed to the workers via inhalation and dermal exposure. These contaminants then can escape from the rice mills transported by the rice millers through contaminated shoes, skin, working clothes and vehicles and be brought to their homes. The family members of the workers could get exposed to these contaminants via the take-home exposure.

These routes of exposure can best to be understood through a conceptual model that established by Jones and Burstyn, (2018). In the model, they proposed three major pathways of the exposure; external contamination, internal dose and behaviour change of the workers. Both external and internal dose involve the routes of dermal exposure, inhalation exposure and ingestion exposure. However, this model only focuses on the route without discussing on the effects of exposure. This current study adopted concept of the external exposure which involve inhalation and take-home exposure. The investigated routes of exposure only involve the external exposure, without taking consideration to the internal dose since there is no evidence have been reported regarding exposure to endotoxins. Unlike exposure to pesticides, lead and asbestos, the samples urine and blood were taken since those exposure lead to the internal doses (Betti *et al*., 2017; Coronado *et al*., 2002; Mandić-Rajčević *et al*., 2018).

Endotoxin that are inhaled by the workers enter the respiratory tract and end up in the lungs. Acute symptoms experienced by the exposed workers such as dry cough and dyspnoea, and the symptoms can develop to bronchoconstriction, headache and aching joints after few hours. The cross-shifts decline in FEV_1 over a single day may indicate acute effects undergone by the workers. The chronic effects can be predicted by measuring FEV_1 annually (Health Council of the Netherlands, 2010). However, in this study, the researcher only focusses the measurements on single day occasions pre-shifts and post-shifts of the lung functions.

In terms of take-home or para-occupational exposure, in wider community, the affected groups are the family members of the exposed workers. Para-occupational can be explained as the people who lives together with the exposed workers, get the exposure to the contaminants brought into homes but they are not occupationally exposed. The contamination might be transmitted by holding the children, the hand-washing of the contaminated clothes and inhaling the deposited dusts in the house. However, this study only determined the possible take-home exposure by taking the post-shifts hands wipes of the workers and vacuum home dusts.

Figure 1.1 shows the main idea of this study. In summary, workplaces serve as the source of contaminations and the workers mainly exposed to it through inhalation and dermal exposure. The contaminations were then transmitted to the family members via take-home exposure. In this case, only hands wipe and vacuum home dusts were taken as parameter samples to determine on the possible contaminations. Note that, the respiratory and lung function tests assessment only involves the rice millers, without checking the respiratory conditions since only possible route of exposure were evaluated.

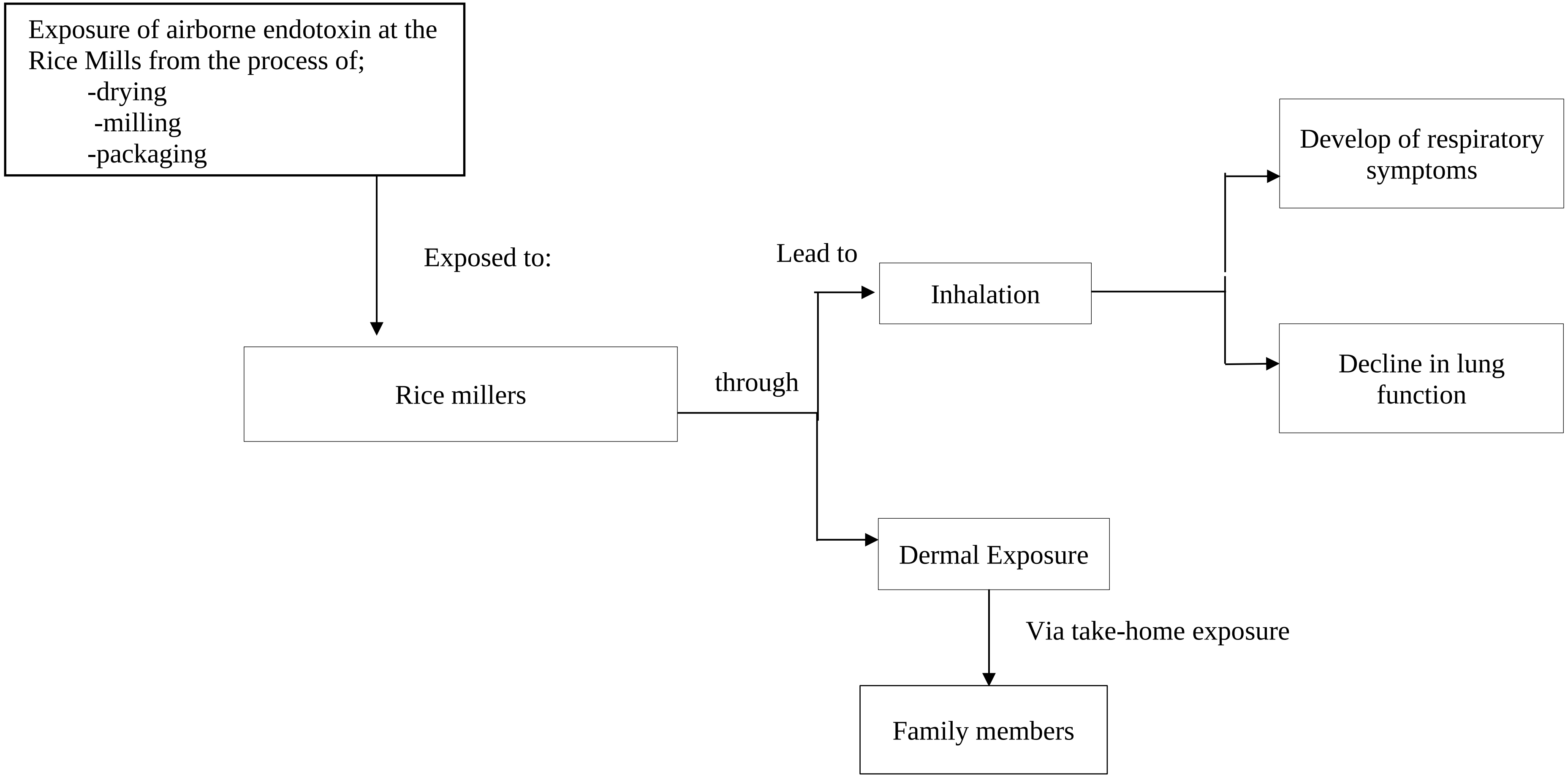

Figure 1.1 Flow chart showing the conceptual framework of route of exposure that lead to adverse respiratory effects

1.5 Justification and Benefit of Study

The significant of this study is to determine the exposure mechanisms of endotoxin in rice dust among rice millers from workplaces to homes. From this study, the level of endotoxin of the exposed workers would be determined. The respiratory symptoms experienced by the workers as well as the lung function were also identified. Hence, this study would propose a proper way to reduce and lessen the exposure of rice dust directly to the workers by recommending suitable control measures to prevent the inhalation of biological agents such as endotoxin among the workers during working. In addition, this study would suggest the workers to change their clothes and taking shower at the premises before going home to reduce the take-home exposure which in turn might affect the family members of the workers.

The findings of this study will benefit the society considering that the health and wellbeing of the workers as well as their family members which are the main concern that could be improved. The increasing cases towards the occupational and take-home exposure justifies that the need of reducing exposure at the sources and avoid the route of exposure to the family members. The premises were recommended to supply correct respirators (N95) instead of surgical face mask or using clothes as well as to provide facilities such as shower and clothing changing room, for the workers to change to street clothes after working session.

CHAPTER 2

LITERATURE REVIEW

This literature review was conducted according to the method of systematic review. The search engine used mostly PubMed Advance search, Google Scholar, Elsevier, Google books and Science Direct. The keywords used for example in PubMed advanced was “endotoxin” AND “rice mill”, “endotoxin” AND “occupational”, “endotoxin” AND “agricultural”, then “occupational” AND “respiratory symptoms” and “occupational” AND “lung function”. The terms such as “take-home”, “para-occupational”, “environmental” also used to search for the take-home exposure sub-topic. The results then adjusted to year range 2010 onwards. However, in some cases, those studies that related to this current study also included in this thesis. For the terms, operational definition and process mechanism of action Google Books were used for better understanding. The articles found were screened according to the title and abstract to make sure the articles were related to this current study.

2.1 Exploring Endotoxin Exposure

Firstly, before it is proceeded with the elaboration on the endotoxin and rice dust, it is better to define the terms of the “particulate matter” (PM). PM is a mixture of solid particles and liquid droplets which is found in the air (Environmental Protection Agency United States, 2018). According to Health and Safety Executive, (2014) as stated in ISO 7708 or BS EN 481, the aerosol size fraction can be divided into three fractions; inhalable, thoracic and respirable. Inhalable fraction is the airborne materials which enters the nose and mouth at the time of breathing and can be deposited in the respiratory tract. For thoracic fraction inhaled it will be deposited beyond the larynx. Whereas the respirable fraction is the airborne materials which penetrates to the lower gas exchange region of the lung when inhaled.

The study on the exposure to endotoxin have been broadly published. Many studies have reported the presence of endotoxin in environment; indoor environment and which the interest had later grown to occupational setting. Rylander, (2002) focused in studying the endotoxin in the environment had lists up the presence of endotoxin found in homes, in agriculture, in waste as well as in the industry. Then, Spaan *et al*., (2006) focuses the research of endotoxin exploring larger samples size in a wide range of agricultural industries. From these two studies, it can be concluded that high level of endotoxins was detectable in the environment. With the structure of endotoxin which is stable in nature, endotoxins can withstand in the environment even in extreme condition.

Apart from that, this current study focusses on endotoxin exposure in the occupational settings. Table 2.1 summaries the latest studies reported from 2010 to 2019 on the endotoxin exposure as well as the level of endotoxin concentration detected in workplace from various countries. The industry includes rice mill industry, wood dust industry, textile industry, dairy, coffee and faecal sludge.

In spite of various occupational exposure to endotoxin, this study focuses on the rice industry. In Malaysia, rice industry is one of the leading products which dominated by Malaysia in agricultural industry apart from palm oil, rubber and coconut (Sawe, 2017).

Table 2.1 Studies of Endotoxin Exposure in Workplace Exposure

Type of Occupational Exposure	Author (year)	Country	Work Population	Endotoxins Concentration (EU/m^3)
Rice Mill	Dimjati Lusno *et al.*, (2018)	Indonesia	Paddy milling operators (n=18)	Average EU/m^3 concentration: 232.22±18.41
	Lamawuran *et al*., (2015)	Indonesia	Rice mill operator (n=11)	Average concentration: 56.36±5.83 EU/m^3
	Hendrarinata *et al.*, (2015)	Indonesia	Rice mill operator (n=11)	Average concentration: 91.1±21.87 EU/m^3
Wood dust	Rianto *et al.*, (2016)	Indonesia	Wood furniture workers (n=12)	Mean concentration of endotoxin: 2.18 ng/m^3
Textile/Cotton	Ghani *et al*., (2016)	Pakistan	Exposed workers (n=100)	Range for mean of endotoxin in the workplace: 40-300 EU/m^3
	Paudyal *et al*., (2011)	Nepal	Full shift Workers (n=114)	GM: 2160 EU/m^3
	Shi *et al*., (2010)	China	Exposed workers (n=447) from 1981 to 2006 at 5-years intervals.	Cumulative endotoxin exposure 52,820 ± 45,507 EU/m^3-years
Dairy	Arteaga *et al*., (2015)	California	Exposed Workers (n=185)	GM: 331.5EU/m^3 to inhalable particulate matter 12.5EU/m^3 to particulate matter
Coffee	Sakwari *et al*., (2013)	Tanzania	Exposed Workers (n=154)	Robusta Factory: 10 800 EU/m^3 Arabica Factory: 1400 EU/m^3
Faecal Sludge	Sklar *et al*., (2019)	Rwanda	Exposed Workers (n= 13)	Range of personal samples: 2.6 EU/m^3 to 900 EU/m^3

2.2 Rice Production and Consumption

Rice is said to be the staple food of nearly half of the world population (Chauhan *et al*., 2017). Most of the parts in the world grown this kind of crop namely Asia, Africa, Australia, Europe, North America and South America except for icy continents of Antarctica (Chauhan *et al*., 2017). Increasing population of the world lead to the increase demand of rice production as estimated by the United Nations that the global population reach up to 7.5 billion, with the Asia make up the biggest portion of the world (Omar *et al*., 2019).

In order to meet the demand, the worldwide rice production is elevated to over 495 million metric tons in 2018 and 2019 instead of 700 metric tons milled in the previous years (Shahbandeh, 2019). Global rice consumption increased about 89% from 437 million metric tons to 490 million metric tons in the year 2018 to 2019. The leading country consuming rice are China, India and Indonesia (Shahbandeh, 2019).

Asia is largest part of the world that produces and consumes rice which accounts about 90% of the world production and consumption. Currently, the population in Asian country has reached about 4.463 billion in 2020. The increasing population leading to the increase demand of consumption of rice. Abdullah *et al*., (2006) estimated the increasing net demand of rice of over 481.9 million tons by 2025 and 525 million tons by 2050.

2.2.1 Milling Process from Paddy to Rice

Rice specifically *Oryza* species is the member of grain or grass family. Among 22 species discovered, only two species were cultivated where species of *Oryza Glaberrina* is grown in West Africa and *Oryza Sativa* is cultivated in the rest of the world (Hatami *et al*., 2015). The structure of paddy grain consists of caryopsis which enclosed in a tough husk or hull. The hull acts as a protection against insect infestation and fungal damage. This husk is not edible and in the form of woody structure. The edible part of the paddy is the endosperm. The layer that covering the endosperm is called pericarp which consists of epicarp, cross cell and cuticle. The next layer enclosing endosperm is testa or seed coat, followed by nucellus and aleurone layers. Apart from endosperm, there is embryo attached to the one end of the endosperm (Manickavasagan *et al*., 2017).

Milling process is the process where the paddy is transformed into edible form to be consumed by humans. Milling process involves various processes and stages depending on the objective of the milling process. Roughly, milling process can be divided into two main levels; village level or traditional method and modern commercial industrial level. Village level is normally for local consumption, where simple milling stages and simple machinery is used; namely hand milling and hulling process. Industrially, milling process involves multi stages of process depending on the objectives of the milling and the end-product acquire for commercial purpose (Dhankhar, 2014; Tangpinijkul, 2010).

In industrial scale, the process started with the drying of the paddy, since the paddy acquire moisture through gelatinization of starch, drying process is to remove the moisture content up to 45-50% (Ali and Pandya, 1974) leaving best milled rice at 13-14% of moisture content (International Rice Research Institute, 2015) or 14-16% dried to obtain desirable milling and storing properties (Manickavasagan *et al*., 2017). The best temperature should not be exceeded 45ºC throughout the drying process (International Rice Research Institute, 2015). After the drying process, the paddy undergoes pre-cleaning process where the foreign materials (straw, weed seeds, soil and other inert materials) are removed. The pre-cleaning process is important since the foreign materials will affect the efficiency of the of the huller and milling recovery (Gummert, 2010). The dehulling or dehusking process is to remove the husk layer from the paddy leaving brown rice. The destoning process is to separate the small stones from the brown rice. The paddy is then undergone separation process to separate unhusked paddy with the brown rice. The final step of rice milling involves whitening and polishing of the rice to improve the appearance of the rice and storage of the white rice (Tangpinijkul, 2010). The summary of transformation of paddy which undergo milling process can be best be understand through the flowchart as shown in Figure 2.1 while the process in rice mill factory is illustrated in Figure 2.2 (Padiberas Nasional Berhad, 2019).

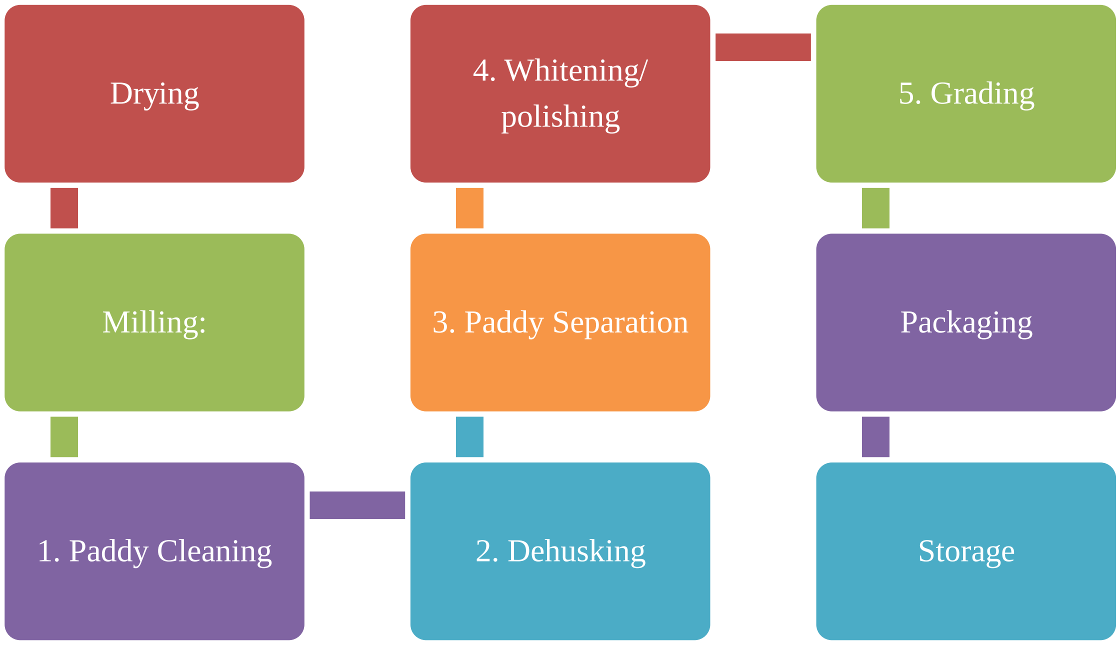

Figure 2.1 Rice Processing in rice mill adapted from Padiberas Nasional Berhad, (2019)

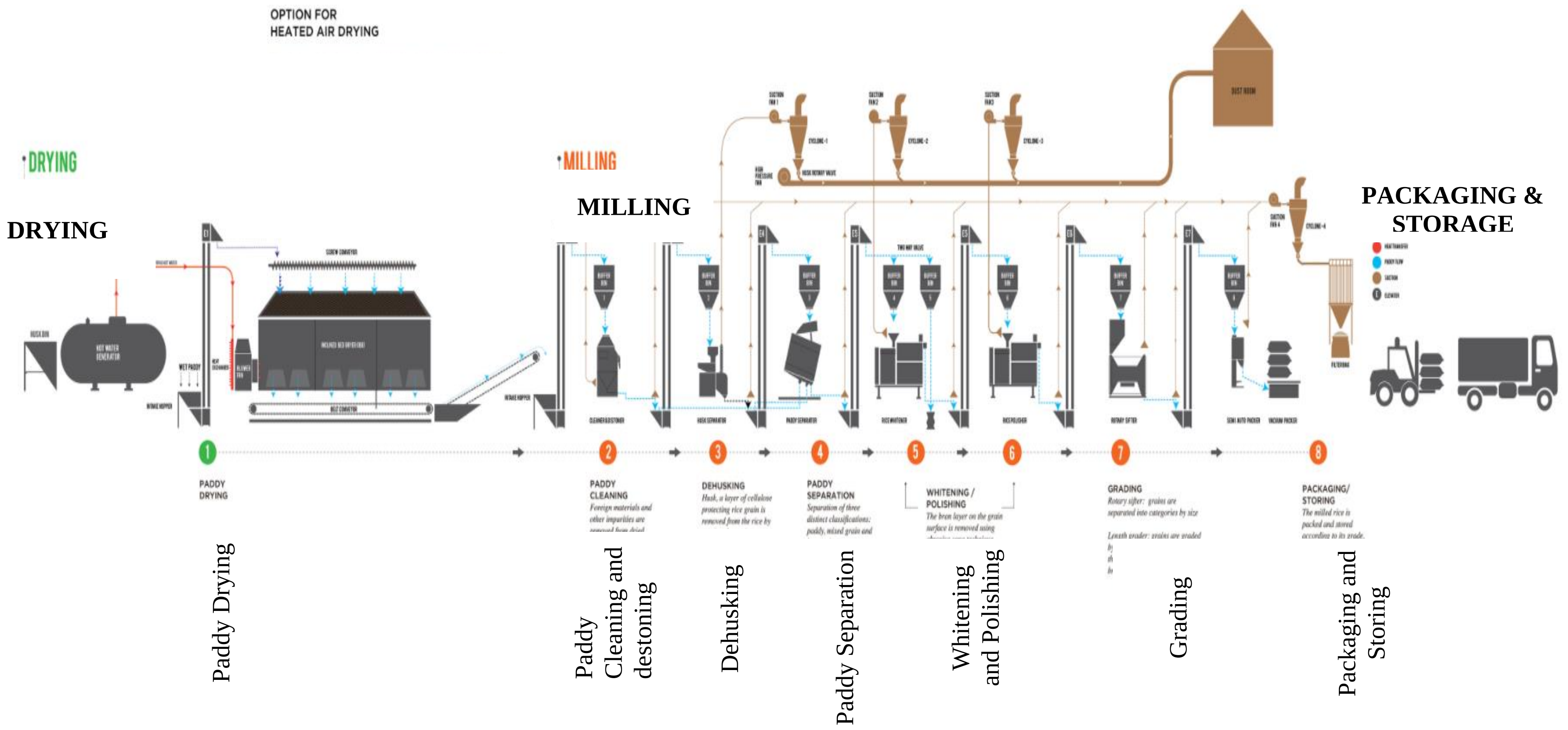

Figure 2.2 Drying and milling process in rice mill adapted from Padiberas Nasional Berhad, (2019)

2.2.2 Dust Emitted during Rice Processing

The rice milling process produces large amount of dust especially during the paddy season. Hence, the workers are more likely to be exposed to the organic rice dust especially those who are in charged with the milling process. A previous study by Dewangan and Patil, (2014) showed that the respirable and total dust of the rice mills in India were 8.22 mg/m^3 and 81.05 mg/m^3 respectively which exceeded the permissible exposure limit (PEL) of Indian Union Ministry of Labour.

Another study in Thailand at a local rice mill showed an average total dust of 6.76 mg/m^3 and 9.79 mg/m^3 for both wooden rice mill and iron rice mill, respectively (Batsungneon and Kulworawanichpong, 2011). The paddy pouring station showed the highest amount of dust emitted; 22.44 mg/m^3 in wooden rice mill and 25.0 mg/m^3 in the iron rice mill. Even though it was a small-scale industry, the total dust emitted was exceeded the standard level of allowance by the Ministry of Resources and Environment in Thailand. In conclusion, rice processing in the rice mills emitted large amount of dusts, potentially above the recommended limit.

2.3 Source of Endotoxin Exposure

2.3.1 Occupational Exposure

As described previously, the process in the rice mills produced large amount of dust and the dust is potentially be contaminated with endotoxin. The microbiological growth may occur during culturing, processing, storage and transport of agricultural products (Spaan *et al*., 2006). The cell wall of the bacterial breakdown and the inner parts become airborne. The major route of exposure to endotoxin is said to be through inhalation. The inhalation of endotoxin had caused various effects to respiratory health of the workers that will discuss further in next section.

2.3.2 Para-occupational Exposure

Apart from inhalation route at workplaces, another route of interest is take-home exposure. The workers also might 'transport' the contamination at the workplace to their homes. The concerns towards the 'take-home' exposures had become a global attention since many cases were reported involving children and family members of the workers in some premises.

On the 26th October 1992, National Institute of Occupational Safety and Health (NIOSH), has enacted an act enactment namely The Workers' Family Protection Act (29 U.S.C. 671a) as section 209 of Public Law 102-522. The main objective of this act is to protect the family members of the workers from the hazardous chemicals and infectious agents were brought from workplace to home (The National Institute for Occupational Safety and Health, 2012).

This current study focusses on the direct and physically contamination. Direct contamination occurred when the workers leave the workplace with the contaminated clothes, shoes, skin and vehicles. The hazards from the workplace escape 'driven' by the workers into the home. Previous study found detectable of contaminants on their hands, forehead and shoes and that these allergens are present in their cars (steering wheel, footwear, driver's seat) with the highest levels of contamination being seen on their shoes and car footwell/driver's seat (Tagiyeva et al., 2012). Holding and playing with their children once arrived home without washing hands or taking bath causing the hazards on the clothes transferred directly to their children. In addition, the deposited dusts in the home (Noonan, 2017) together with the exploratory behaviour and frequent hand-to-mouth activity of the children increase the potential of contamination among the children (Roberts and Karr, 2012).

Another route of exposure is through hand-washing of the working clothes contaminated with the chemicals and infectious agents from the workplace. These contaminations are transmitted through the skin (Blanco-Muñoz and Lacasaña, 2011). An experimental study conducted by Sahmel *et al*., (2014) on the loading, handling and shaking out the clothes contaminated with chrysotile asbestos found person involved with these activities exposed cumulatively about 0.00044-0.105 f/cc year.

Previous study on lead exposure among the children, a study reported that having fathers who worked at the smelter were one of the main determinants and detectable of increase potential four times of the elevated blood lead level, (BLL) in their children Mandić-Rajčević *et al*., (2018). Rinsky *et al*., (2018) has confirmed that take-home exposure is the sources in their investigation among the children having elevated BLL.

The vehicles used by the workers are one of the important sources of take-home exposure especially the vehicles were park near the operating plants with the windows opened. Fenske *et al*., (2013) suggested the workers to regularly vacuum the vehicles could reduce the pesticides deposited thus lessen the potential of take-home exposure. Apart from that, they also suggested the employers to provide changing areas, storage facilities as well as proper training on safety handling of pesticide.

The adverse health involving take-home exposure from the parents or workers who's occupationally exposed to the dust at the workplaces living together with other family members has been proven as shown in a systematic review by Donovan *et al*., (2012). They summed up reported cases on asbestos related diseases caused by potential take-home route. Another report by Newman *et al*., (2015) summarised on cases of elevated lead in children's blood caused by the take-home exposure from the parents. Previous study also assessed on the possible exposure pathways for organophosphate for children in agricultural communities, indicated one of the possible pathways was the parental take-home (Fenske *et al.,* 2000).

Figure 2.3 summarises the potential of take-home exposure pathway. The route labelled as number 1 showed the contamination through the vehicles of the workers. For the second route, the main source of contamination is the workers themselves. Fathers or mothers playing with children once arrived from work (2a), the exploratory behaviour of the children (2b) and hand washing of the working clothes contaminated with the contaminants (2c) are the potential exposure of the contaminants to the family members of the workers.

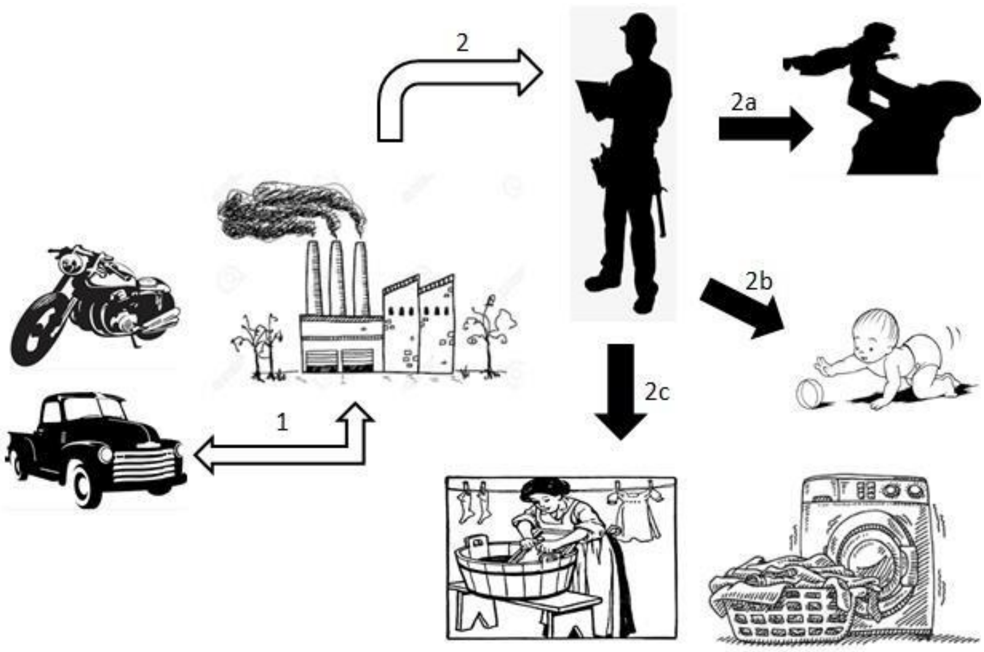

Figure 2.3 Possible Take-Home Exposure Pathway

2.4 From Exposure to Health Effect

2.4.1 Physical Characteristics of Endotoxins

Before further exploring on the health effects caused by endotoxin, it is essential for us to understand the physical, chemical and biological characteristics of endotoxin since virulence of toxicity of endotoxin depends on the characteristics of endotoxins.

Endotoxins are first discovered in the late nineteenth century when it was revealed that heat-killed cholera bacteria were themselves are toxic, instead of causing toxicity by secreting a product from the living organisms. The secretion product is called exotoxins whereas the toxic materials themselves are called endotoxins. Endotoxins fall into the category of lipopolysaccharide (LPS) which is the major component of the cell wall. People usually use the word of endotoxin and LPS interchangeably (Wang and Quinn, 2010).

Endotoxins primarily consists of three major parts; Lipid A which consists of hydrophobic segments and inner core, polysaccharide chains which is the central part, locates in the outer leaflet of the outer membrane and have the hydrophilic properties, the intermediate layer of endotoxins and lastly specific O-antigen. This core sugars and O-antigen repeats are displayed on the surface of the bacteria. The so-called LPS is because of polysaccharide chain linked to a lipid moiety referred to as Lipid A and known to be responsible for the toxic effect of infections with Gram-negative bacteria. The toxicity of endotoxins can be diverged from a bacterium to a bacterium or even from cell to cell (Boss and Day, 2003).

Apart from that, the characteristics of endotoxins include ubiquitous in nature physically and chemically and has potent toxicity. In addition, they are durable nature, naturally high pro-inflammatory capacity and heat stable (Spaan *et al.*, 2006).

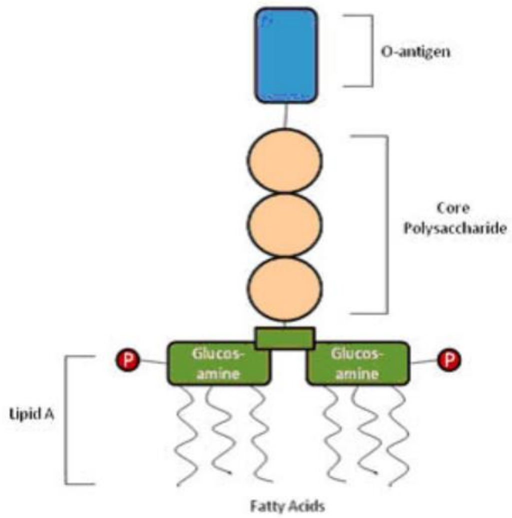

Figure 2.4 Structure of endotoxin adapted from Sigma-Aldrich, (2020)

2.4.2 Mechanism of Endotoxin Action

The entry of the biological agents into the body includes by oral digestion, inhalation, dermal absorption and injection. Route of exposure to these biological agents by breathing the air that contains biological agents and penetration of the skin by the biological agents. The introduction of endotoxin into the body by any means route of exposure simply have the similar mechanisms. However, the response is differ based on the how much the chemical absorbed by the organisms, the potentials of the bacteria to reach the target organs, environmental and activity factors that might exposure the subjects to the endotoxin, the duration and frequency exposure (Boss and Day, 2003).

Once endotoxin is introduced into the body, the host cells which are targeted by the endotoxin develop defense mechanisms. The first line of the defense mechanisms binds of endotoxin to lipopolysaccharide binding protein in the serum and transfer to cluster of differentiation, (CD 14) on the cell membrane. The endotoxin then transfers CD 14 to lymphocyte antigen 96 or known as MD 2, which associates with Toll-like receptor-4, (TLR-4). The TLR-4 is a transmembrane protein that is present is several types of the immune systems includes macrophages and dendritic cells. Once TLR-4 binds with endotoxins, it undergoes conformational changes which triggers a signalling cascade inside the cells. The cells then secrete cytokines and nitric acid that lead to sepsis. Sepsis characterized by a whole-body inflammatory state may lead to low blood pressure, multiple organ dysfunction syndrome, systemic inflammatory response syndrome (Jaffer et al., 2010) and even death (Xiaoyuan *et al*., 2010)

Exposure to endotoxins lead to various health effects. Previous study by Thorn and Rylander, in 1998 on the healthy subjects inhaled endotoxin lead to lowered in FEV_1 and FVC values which were significantly decrease within 24 hours inhaled of endotoxin compared to before the tests. In addition to that, through questionnaire the subjects also complained about a few symptoms that developed after inhalation to endotoxin such as irritation in the throat, dry cough, breathlessness, unusual tiredness, headache and heaviness in the head occurred significantly than before tests. In addition, increase level of C-reactive protein, (CRP) in blood and increase if eosinophilic cationic protein (ECP) and myeloperoxidase (MPO) in serum implies markers of non-specific inflammation. Overall, this study suggests that inhalation to endotoxin causes presence of an acute inflammation in the airways, indicated by the increased amounts of inflammatory markers in sputum as well as in the respiratory symptoms.

Another study by Loh *et al*., in 2006 on healthy subjects on the different doses of LPS; 15 µg and 50 µg showed that after inhaling endotoxin, the subjects experienced flu-like symptoms with the addition headache, chills and muscle ache. These symptoms are acute since it occurred as early as two hours and resolved within by 24 hours. In addition, the subjects experienced elevation in temperature at 4^{th} hour onwards. After six hours of inhalation, total cell and neutrophil counts in induced sputum as well as total white cell and neutrophil counts in peripheral blood showed significantly increase. This study concluded that, in higher doses of endotoxin, the systemic effects were greater compared lower doses of endotoxin. In addition to that, the inflammatory nature was predominantly neutrophilic and more prominent in airway rather than in blood. Therefore, this current study focuses on the respiratory symptoms experienced by the rice workers as well as decline in lung function tests.

2.4.3 Respiratory Effects

Occupational exposure to organic dusts leads to various respiratory effects. Previous study among rice millers in India found that the workers complained several types of respiratory disorder like phlegm (40.8%), dyspnoea (44.2%), chest tightness (26.7%), cough (21.7%) and nose irritation (27.5%) (Ghosh *et al*., 2014). Another study among flour mill workers in Egypt complained on the respiratory such as cough, expectoration, wheezing and shortness of breath higher among exposed compared to non-exposed (Mahmoud, 2011). Whereas, a longitudinal study in Germany among compost workers found compost workers reported a significantly higher prevalence of mucosal membrane irritation (MMI) of the eyes and upper airways than control subjects in addition to conjunctivitis which was diagnosed among the workers. (Bünger *et al*., 2007).

However, these presented studies did not relate the exposure with the endotoxin. Mayan *et al*., (2002) came out with the hypothesis that respiratory