RESPIRATORY EFFECTS OF AFLATOXIN B1 AND RISK OF WORKPLACE TO HOME EXPOSURE AMONG SELECTED RICE MILLERS IN MALAYSIA

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by

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LIST OF SYMBOLS

&	Ampersand
˚C	Celcius
cm/h	Centimeter per hour
=	Equal to
<	Less than
≤	Less than or equal to
µg/dL	Microgram per decilitre
µg/kg	Microgram per kilogram
$\mu g/m^3$	Microgram per metre cube
mg/m^3	Microgram per metre cube
>	More than
≥	More than or equal to
ng/kg	Nanogram per kilogram
ng/m^3	Nanogram per metre cube
ng/ml	Nanogram per millilitre

%	Percentage
±	Plus-minus sign
a_w	Water activity

LIST OF ABBREVIATIONS

AFB1	Aflatoxin B1
AFG1	Aflatoxin G1
AFG2	Aflatoxin G2
AFM1	Aflatoxin M1
AFM2	Aflatoxin M1
AF-Alb	Aflatoxin-albumin
BERNAS	Beras Nasional
BMRC	British Medical Research Council
BAL	Bronchoalveolar lavage
CMI	Cell-mediated immunity
CDC	Center for Disease Control and Prevention
COPD	Chronic obstructive pulmonary disease
$CD3^+$	Cluster of differentiation 3 (T-cell co-receptor)
CI	Confidence interval
DNA	Deoxyribonucleic Acid
DOSH	Department of Occupational Safety and Health
ELISA	Enzyme-Linked Immunosorbent Assay
et al.	*Et al*ia (and others)
ECRHS	European Community Respiratory Health Survey
FEV1	Forced expiratory volume
FVC	Forced volume capacity
FEV1/FVC	Forced volume capacity and forced expiratory volume ratio

GM	Geometric Mean
HBsAg	Hepatitis B surface antigen
HCC	Hepatocellular carcinoma
HEPA	High-efficiency particulate air
HPLC	High-Performance Liquid Chromatography
IOM	Institute of Medicine
IARC	International Agency for Research on Cancer
JEPeM	Jawatankuasa Etika Penyelidikan Manusia
MRCQ	Medical Research Council Questionnaire
NER	Nucleotide Excision Repair
NIOSH	National Institute for Occupational Safety and Health
OR	Odd ratio
PEF	Peak expiratory flow
PEFR	Peak Expiratory Flow Rate
PPE	Personal Protective Equipment
PBST	Phosphate buffer tween-20
ROS	Reactive Oxygen Species
spp.	Several species
SGRQ	St. George Respiratory Questionnaire
SD	Standard deviation
TWA	Time-weighted average
TPMA	Tumour promoter 12-tetradecanoyl phorbol myristate acetate
UNDP	United Nations Development Programme

LIST OF APPENDICES

KESAN RESPIRATORI AKIBAT AFLATOKSIN B1 DAN RISIKO PENDEDAHAN DARI TEMPAT KERJA KE RUMAH DALAM KALANGAN PEKERJA KILANG BERAS TERPILIH DI MALAYSIA

ABSTRAK

Pemprosesan padi menghasilkan habuk mungkin mengandungi toksin kulat disebabkan oleh pengendalian dan penyimpanan padi pada tahap kelembapan tinggi yang tidak sesuai. Aflatoxin B1 (AFB1) adalah yang paling kuat antara aflatoksin dan karsinogenik terhadap manusia jika ditelan atau disedut serta boleh menyebabkan barah hepatoselular atau barah paru-paru. Kajian ini bertujuan untuk menilai pendedahan AFB1 dan kesannya terhadap saluran pernafasan dalam kalangan pekerja kilang beras yang terpilih di Malaysia. Pendedahan debu di kawasan kilang dan terhadap pekerja (individu) dinilai menggunakan penyampel yang terdiri daripada penapis kaca mikro gentian, penyampel IOM dan Pam Persampelan Udara Individu GilAir Plus. Pam tersebut dipakai selama lapan jam bekerja, dipasang pada tali pinggang pekerja. Ujian fungsi paru-paru dilakukan sebelum dan selepas shif. Telapak tangan kedua-dua tangan pekerja disapu dengan kapas steril yang dilembapkan dengan larutan 'Phosphate Buffered Saline Tween-20' (PBST), sebelum dan selepas shif. Persampelan debu rumah dilakukan dengan menyedut debu ruang tamu menggunakan pengumpul DUSTREAM yang mengandungi penapis jaringan nilon yang dipasang pada hos penyedut hampagas. Dapatan daripada sampel tersebut kemudiannya dibandingkan dengan kumpulan kawalan yang merupakan pekerja pentadbiran USM. Borang soal selidik diberikan kepada peserta kajian untuk mengumpul maklumat mengenai data sosiodemografi, perincian pekerjaan, kepatuhan peralatan perlindungan diri (PPE), amalan di tempat kerja, gejala pernafasan dan faktor-faktor yang

menyebabkan pembawaan AFB1 ke rumah. Secara keseluruhan, terdapat 115 subjek yang mengambil bahagian dalam kajian ini, 77.5% daripadanya berbangsa Melayu. AFB1 dikesan dalam 14.1% (n = 10) sampel debu udara (median: 0.16, IQR: 0.14-0.90). Hasil sapuan tangan selepas shif didapati bahawa dua pekerja kilang beras (13.0%, n = 2) positif dengan AFB1 (median: 0.24 ng / ml, IQR: 0.24-0.27 ng/ml). Walau bagaimanapun, AFB1 tidak dapat dikesan pada swab tangan selepas shif dalam kalangan kumpulan kawalan (<LOQ: 0.24 ng/ml). Tiada perbezaan yang signifikan ditunjukkan oleh fungsi paru-paru (selepas shif) antara pekerja kilang beras dengan kumpulan kawalan (FVC: p = 0.911, FEV1: p = 0.637, FEV1 / FVC: p = 0.385, PEF: p = 0.160) setelah 'jantina' dan 'tabiat merokok' diletakkan sebagai pengeliru (confounder), tahap debu rumah AFB1 dalam kalangan pekerja kilang beras ialah 0.18 ng/kg (IQR: 0.08 - 8.32). Tiada perbezaan yang signifikan bagi tahap AFB1 dalam debu rumah antara kedua-dua kumpulan. Sebagai kesimpulan, tahap AFB1 yang dikesan di kilang beras (dalam penapis udara dan pada sapuan tangan) lebih rendah daripada had yang dibenarkan (30 ng/m^3). Kesannya, tiada korelasi yang signifikan dapat dibuktikan antara tahap AFB1 di tempat kerja dengan di rumah. Oleh itu, kehadiran AFB1 di rumah mungkin dipengaruhi oleh faktor persekitaran seperti aktiviti manusia di rumah. Walaubagaimanapun, kesan kumulatif boleh menimbulkan risiko selama bertahun-tahun, jadi langkah-langkah kawalan seperti amalan kebersihan di tempat kerja dan pencegahan pembawaan ke rumah amat disyorkan untuk mengurangkan tahap pendedahan terhadap AFB1 dalam kalangan pekerja dan ahli keluarga mereka.

RESPIRATORY EFFECTS OF AFLATOXIN B1 AND RISK OF WORKPLACE TO HOME EXPOSURE AMONG SELECTED RICE MILLERS IN MALAYSIA

ABSTRACT

Rice milling process produces a huge amount of dust which may contain fungal toxins due to improper handling and storage at high humidity levels. Aflatoxin B1 (AFB1) is the most potent among the aflatoxins and carcinogenic to humans when ingested or inhaled that might cause hepatocellular cancer or lung cancer. This study aims to assess the exposure of AFB1 and its respiratory effects among selected rice millers in Malaysia. Area and personal dust exposure were evaluated using a sampling train consist of a glass microfibre filter, IOM sampler and GilAir Plus Personal Air Sampling Pump. The pump was worn for eight working hours, attached to the workers' belt. Lung function test was performed pre and post-shift. The palms of both hands of the workers were swabbed with sterile cotton pads wetted with Phosphate Buffered Saline Tween-20 solution (PBST), pre and post-shift. Home dust sampling was done by vacuuming the living room using a DUSTREAM collector containing mesh nylon filter attached to a vacuum cleaner. The results were then compared with the control group involving USM administration workers. Questionnaires were given to collect information on sociodemographic data, occupational details, personal protection equipment (PPE) compliance, workplace practices, respiratory symptoms and factors that lead to take-home exposure to AFB1. Altogether, there were 115 subjects participated in the study, 77.5% of them are Malay. AFB1 was detected in 14.1% (n = 10) of personal airborne dust samples (median: 0.16, IQR: 0.14-0.90). Post-shift hand swab was positive with AFB1 in two rice millers 13.0% (n = 2) (median: 0.24 ng/ml,

IQR: 0.24-0.27 ng/ml). However, AFB1 was non-detected on hand swabs among office workers (<LOQ: 0.24 ng/ml). There was no significant difference was found in post-shift lung function between rice millers and controls (FVC: $p = 0.911$, FEV1: $p = 0.637$, FEV1/FVC: $p = 0.385$, PEF: $p = 0.160$) after controlled for gender and smoking as confounders. The AFB1 home dust level among rice millers was 0.18 ng/kg (IQR: 0.08 – 8.32). There was no significant difference in AFB1 levels in home dust between both groups. To conclude, detected AFB1 at rice mills (airborne filter and hand swabs) were lower than the permissible limit (30 ng/m^3). Consequently, there is no significant correlation between workplace and home AFB1 levels. Thus, the presence of AFB1 at home could be influenced by environmental factors such as human activities at home. Cumulative effects may pose risks over the years so proper control measures such as hygiene practices at work and preventing take-home exposure are highly recommended to reduce the levels of AFB1 among the workers and their family members.

CHAPTER 1

INTRODUCTION

1.1 Study Background

Aflatoxin is a metabolite of *Aspergillus spp.* and is mainly produced by *Aspergillus flavus* and *Aspergillus parasiticus* (Hamid, Tesfamariam, Zhang, & Zhang, 2013). Exposure to aflatoxin could lead to various health effect such as aflatoxicosis (Nurul Adilah & Mohd Redzwan, 2017), pulmonary oedema (Abedi & Talebi, 2015), cerebral oedema (Saleem, Sadia, & Awan, 2017), hepatocellular carcinoma (HCC) (Lai, Mo, Yang, He, & Xiao, 2014), if exposed. There are various types of aflatoxin species, ranging from aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2), aflatoxin M1 (AFM1) and aflatoxin M2 (AFM2) but AFB1 is the most potent among them. Based on the evidence in humans and animals, AFB1 has been classified as Group 1 carcinogens by International Agency for Research on Cancer [IARC] as it is carcinogenic to humans (Hamid *et al.*, 2013).

In 1960, a disease known as Turkey X emerged in the United States of America (USA) and England where 100,000 turkeys were found mysteriously dead. After investigation, it was concluded that the turkeys had consumed feeds contaminated with aflatoxin (Bbosa, Kitya, & Lubega, 2013). Twenty-eight years later in Malaysia, 13 children had died in Perak state due to acute hepatic encephalopathy after consuming noodles made of wheat flour contaminated with *Aspergillus spp.* fungi (Mohd-redzwan, Jamaluddin, Abd-Mutalib & Ahmad, 2013).

Following the incidents, organisations all over the world had monitored and established permissible levels for aflatoxin in food such as rice, maize and peanuts. For

example, European Commission has set that AFB1 should not exceed 2 μg/kg in rice while World Health Organisation (WHO) has regulated that aflatoxin in rice must not exceed 30 μg/kg (Reddy, Raghavender, Salleh, Reddy & Reddy, 2011). On top of that, research on the factors influencing aflatoxin contamination in food and the mechanism of aflatoxin transfer from animal's food to animal products are growing. Essentially, most research on aflatoxin are food-focused.

It is worth noting that aflatoxin originates from spore-producing fungi. Therefore, aflatoxin may spread through the air. Since it is evident that aflatoxin is present in grains, grain-handling workers are at risk of being exposed to aflatoxins, especially AFB1 (Amal Saad-Hussein *et al.,* 2014; Afsah-Hejri, Jinap, Radu, & Shakibazadeh, 2013; Reddy, Saritha, Reddy, & Muralidharan, 2009). The workers could be occupationally exposed to AFB1 in many ways; by ingestion, dermal exposure and inhalation. Few studies were done to understand the effect of inhaling AFB1 had found that there is a link between AFB1 with lung cancer (He *et al.*, 2006; Kelly, Eaton & Guengrich, 1997; Jakab, Hmieleski, Zarba, Hemenway & Groopman, 1994). Despite that, pieces of literature on AFB1 dermal and inhalation routes of exposure are limited and the underlying mechanisms are not well understood.

In addition to that, exposure to substances at workplace could be transported home. Such was evidenced in studies on take-home exposure of lead, asbestos and pesticide where the substances were at high levels in the homes of exposed workers (Abelmann *et al.*, 2017; Fenske, Lu, Negrete & Galvin, 2013; Mandić-Rajčević *et al.*, 2018; Thompson *et al.*, 2003). For example, a study on take-home exposure of asbestos had shown laundering activity done by family members such as the wife or the children could expose themselves to two types of asbestos; chrysotile and crocidolite (Abelmann *et al.*, 2017). Similarly, it can be hypothesised that there is a possible take-home

exposure of AFB1 in rice dust. Although, currently, there is no data on AFB1 take-home exposure. Therefore, the current study focuses on the workplace to home exposure of AFB1 and its respiratory effects among rice mill workers.

1.2 Problem Statement

Asia is well known as the largest producer and consumer of rice. In 2018, Malaysia had produced 528,059 metric tons of rice (Malaysian Open Data Portal, 2019). The process to produce rice includes milling that generates a substantial amount of dust. Long-term exposure to rice dust can be detrimental i.e. chest tightness, chronic cough and allergy reactions among rice millers as dust may contain silica, bacteria and fungi with their metabolites. One of the most dangerous fungal metabolites is aflatoxin, particularly AFB1. Aflatoxin is produced by fungal spores. It was found that rice dust had the highest aflatoxin content among other dust samples (Sales & Yoshizawa, 2005). Rice millers can be exposed to AFB1 by inhalation, skin absorption and ingestion. Nevertheless, most studies focused on exposure to AFB1 via ingestion while studies on other routes of exposure are limited. Inhalation of fungal spores in low doses may induce asthma in a sensitive person while in high doses, it can trigger alveolitis (Li & Yang, 2004; Wallenbeck *et al.*, 1991). Rice millers may be exposed to AFB1 by dermal contact during operating machines and handling rice. Dermal contact can also lead to ingestion if rice millers do not properly wash their hands before eating. Additionally, fungal spores containing AFB1 may be transmitted to rice millers' clothes which will then be carried home, causing take-home exposure (Jones & Burnstyn, 2018). Aside from that, other factors such as environmental factors (Cheng *et al.*, 2018; Rasmussen *et al.,* 2013; Magan & Aldred, 2007), vehicles used to go to work (Noonan, 2017; Fenske *et al.,* 2013; Tagiyeva, Anua, Semple, Dick, & Devereux, 2012) and activities

around the house (Górny 2004; Górny *et al.* 2002; Thrasher and Crawley, 2009) may also contribute to take-home exposure. Take-home exposure of lead (Gottesfeld, Tirima, Shehu Mohammed, Fotso & Nota 2019; Mandić-Rajčević *et al.* 2018; Rinsky *et al.* 2018), asbestos (Abelmann *et al.,* 2017; Noonan, 2017; Sahmel *et al.* 2014) and pesticides (López-Gálvez *et al.* 2019; Lu *et al.* 2000; Thompson *et al.* 2014) has been well-established. However, there is a lack of studies on take-home exposure on AFB1. The occurrence of occupational exposure to AFB1 at rice mills may indicate that there is a high chance that take-home pathway of AFB1 might also take place. Thus, this study aims to explore workplace to home pathway of AFB1 and how the respiratory function of rice mill workers are affected by it.

1.3 Study Objectives

1.3.1 General Objective

To study the workplace to home exposure of AFB1 and its respiratory effects among rice mill workers.

1.3.2 Specific Objectives

1. To determine the area and personal airborne AFB1 concentration (ng/m^3) among exposed workers and its association with work factors.
2. To determine and compare the AFB1 concentration of post-shift swabs (ng/ml) and home dust (ng/g) between exposed and controls.
3. To determine and compare the lung function values (forced vital capacity (FVC), forced expiratory value in 1 second (FEV1), FEV1/FVC ratio, and peak expiratory flow rate (PEFR)) and respiratory symptoms between exposed and controls.

4. To correlate the AFB1 concentration of personal airborne filters, personal hand swabs and home dust with the lung function test among exposed workers and controls.

1.4 Study Hypotheses

1. There is a significant association between personal airborne AFB1 concentrations among exposed workers with work factors.
2. There is a significant difference in AFB1 concentration of post-shift swabs and home dust between exposed workers and controls.
3. There is a significant difference in lung function values and respiratory symptoms between exposed workers and controls.
4. There is a significant correlation between the AFB1 concentration of personal airborne filters, personal hand swabs and home dust with the lung function test among exposed workers and controls.

1.5 Significance of Study

Inhalation is one of the main routes of exposure for rice dust but studies in particular on airborne aflatoxin is scarce (Saad-Hussein *et al.*, 2016) which is why this research seeks to explore the take-home pathway of AFB1 and how the exposure will adversely affect rice mill workers' respiratory health. The finding of this study will give insight to related organisations and authorities on the current level of AFB1 exposure at rice mills in Malaysia so that action would be taken towards the betterment of the workplace condition. With the information from this study, hopefully, rice mill companies will be stricter at implementing the hierarchy of control such as engineering control, administrative control and personal protective equipment (PPE) (Department of Occupational Safety & Health [DOSH], 2014) although PPE is the last resort in the

hierarchy, to reduce the exposure to AFB1. Apart from that, this study will help to fill the gap in understanding the take-home pathway of AFB1 with anticipation that it would be the foundation for future research in this area.

CHAPTER 2

LITERATURE REVIEW

2.1 Rice Mills in Malaysia

In Malaysia, BERNAS is a big company that dominates rice purchasing and production. Annually, BERNAS produces about 270,000 metric tonnes of rice in the country (BERNAS, 2020). Apart from BERNAS, there are other small-to-medium Enterprises (SMEs) operating in Malaysia. SMEs are defined based on either sales turnover or the number of full-time employees. There are three sectors classifications; a) Manufacturing, b) Services, c) Others. Rice milling falls under primary agriculture, of the 'Others' category. Table 2.1 shows the operation size of small and medium enterprises (SMEs).

Table 2.1 The operation size of Small and Medium Enterprises (SMEs)

Category	Micro	Small	Medium
Manufacturing	Sales turnover of less than RM 300000 OR full-time employees less than 5	Sales turnover from RM300000 to less than RM 15 million OR full-time employees from 5 to less than 75	Sales turnover from RM 15 million to not exceeding RM 50 million OR full-time employees from 75 to not exceeding 200
Services & Other Sectors	Sales turnover of less than RM 300000 OR full-time employees less than 5	Sales turnover from RM300000 to less than RM 3 million OR full-time employees from 5 to less than 30	Sales turnover from RM 3 million to not exceeding RM 20 million OR full-time employees from 30 to not exceeding 75

From Circular on New Definition of Small and Medium Enterprises (SMEs) by Central Bank of Malaysia, 2013.

The main types of rice in the Malaysian market are white rice, brown unpolished rice and glutinous rice. Classifications criteria are based on length of grain, head rice content, broken rice content and milling degree (BERNAS 2016). Before the commodity is distributed to the market, multistep processes are required to produce rice.

2.2 Rice Milling Process

The rice milling process comprises of eight steps (BERNAS, 2019). Figure 2.1 shows the stages in rice processing. First, wet paddies are dried in inclined bed dryer. The paddies are then cleaned and destoned from impurities like sand, stones and other particles. Next, paddies are passed between rubber rolls to remove husks. The husks will then be separated from paddies by aspiration. The paddies are further separated into paddy, mixed grain and brown rice. The brown rice is whitened and polished to remove bran layer and germ as well as to improve the rice appearance (Pranav & Biswas, 2016). After that, rice is sifted from chipped rice and impurities. Grading takes place to categorise the rice into whole kernel, head rice, large broken, small broken and chips (BERNAS 2016). The final stage is weighing and packaging according to grades. After processing, rice is kept in gunny bags in store facility.

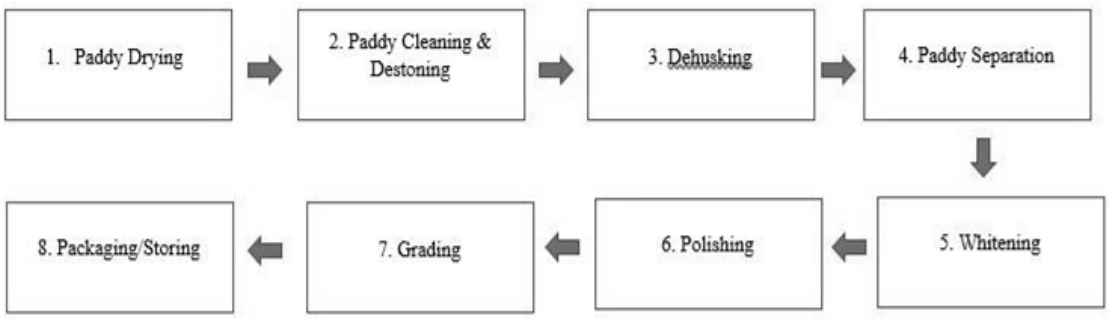

Figure 2.1 Rice Processing (Bernas, 2012)

The rice milling process produced great amount of dust. Dust is solid particles with diameters less than 500 µm. The Swedish National Board of Occupational Safety and Health has set the dust exposure limit of 10 mg/m^3 for normal and 5 mg/m^3 for organic dust (Pranav & Biswas, 2016). On the other hand, the Department of Labour had set the grain dust exposure level at 1.0 x 10^3 $\mu g/m^3$ in 8 hours of time-weighted

average (TWA) and must not go beyond 3.0 x 10^3 μg/m^3 in 10 minutes (Health & Safety Executive, 2007).

In Korea, rice production is indirectly proportional to the rice consumption among Koreans due to preference for alternative food source. Often, rice is stored for lengthy period at rice mills. Prolonged storage might biologically change the rice (Chrastil 1990; Mannaa and Kim 2018). In a previous study, there were evidences of fungal growth in stored rice such as *A. candidus*, *A. fumigatus* and *A. flavus* (Oh *et al.* 2010). Fungal spores are ubiquitous in the environment but prominent exposure to spores is seen in agricultural settings such as waste management and grain handling industry (Hardin, Robbins, Fallah & Kelman, 2009).

Growth of fungi are dependent on warm temperature and high humidity. Malaysia is perfect for the species growth since it is in tropical region (Hejri *et al.*, 2013). *Aspergillus spp.* particularly *Aspergillus flavus* can produce aflatoxin B1 (AFB1) which is potentially dangerous if inhaled (Beizaei *et al.* 2015; He *et al.* 2006).

According to Trucksess, Abbas, Weaver & Shier (2011), 93% of aflatoxins were found in brown rice compared to white rice (28%). When further investigation was made, aflatoxins were found the highest in rice bran (367 μg/kg) and the lowest in rice hulls (39 μg/kg). This is congruent with another study done in Brazil where the highest level of aflatoxin was also found in rice bran (>25 μg/kg). In addition, this study also compares between stationary, intermittent and combined drying process and found that intermittent drying was the most effective way to reduce aflatoxin levels during storage (Prietto *et al.*, 2014). Additionally, in an older but relevant studis done by Sales & Yoshizawa (2005) as well as Purwoko, Hald & Walstrup (1991) also confirmed that rice bran has the highest aflatoxin accumulation. Thus, it could be deduced that the

major aflatoxin source is from the bran and workers who work at whitening and polishing section (bran removal) have higher risk to be exposed to aflatoxins.

2.3 Aflatoxin

2.3.1 Types of Aflatoxin

Aflatoxin is a carcinogenic toxin produced by fungi such as *Aspergillus flavus*, *Aspergillus parasiticus* and rarely in *Aspergillus nomius* (Frisvad, Skouboe & Samson, 2005). There are four major types of aflatoxins; Aflatoxin B1 (AFB1), Aflatoxin B2 (AFB2), Aflatoxin G1 (AFG1) and Aflatoxin G2 (AFG2) where AFB1 is the most potent (Hamid *et al.*, 2013). The International Agency for Research on Cancer (IARC) classified aflatoxin as Class I carcinogen, a category for agents that are carcinogenic to humans and sufficient evidences of carcinogenicity in humans are available (International Agency for Research on Cancer (IARC), 2006). Figure 2.2 shows the chemical structure of AFB1, AFB2, AFG1, AFG2, Aflatoxin M1 (AFM1) and Aflatoxin M2 (AFM2).

Previous studies reported that AFB1 is dominantly observed in food (Adetunji *et al.*, 2014; Ghiasian *et al.*, 2011). Nonetheless, another studies reported that AFB1 and AFG1 concentration was almost similar (Oliveira *et al.*, 2009; Olsen *et al.*, 2008). AFG1 is produced by *Aspergillus parasiticus* and *Aspergillus nomius* and is more potent than AFG2 (Matumba *et al.*, 2014; IARC, 2002). AFM1 and AFM2 are metabolites of AFB1 and AFB2 respectively. AFM1 and AFM2 can be found in milk of mammals that has consumed contaminated feedstuff (Veldman, Meijs, Borggreve & Heeres-Van Der Tol, 1992). AFM1 is less potent than AFB1 and is classified as Group 2 (potentially carcinogenic to humans) but studies showed evidence of immunosuppressive effects on

humans and animals just like AFB1 (Luongo *et al.*, 2014; IARC, 2002; Hsieh & Hsieh, 1987).

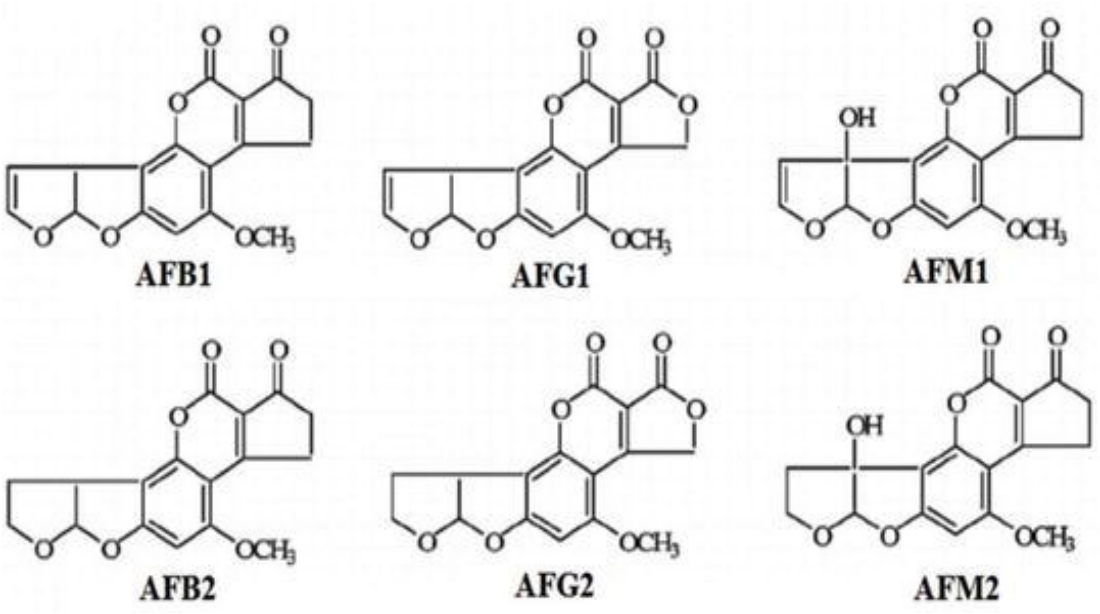

Figure 2.2 The chemical structures of AFB1, AFB2, AFG1, AFG2, AFM1 and AFM2 (Bourais *et al.* 2006).

2.3.2 Aflatoxin Production

There is a list of conditions that influence the growth of *Aspergillus flavus* for example, temperature, water activity (a_w), nutrient source and pH (Klich 2007). Water activity is the ratio between vapour pressure of food and vapour pressure of distilled water (The United States Food and Drug Administration [USFDA], 2014). In simpler words, water activity is the measure of water in food that is unbound to food molecules. The unbound water molecules provide optimal condition for fungal growth.

Similarly, aflatoxin formation is also affected by temperature change. Obrian *et al.*, (2007) showed that the optimum temperature for aflatoxin production is 28 – 30°C but as the temperature hits 37°C, the production declines because fungal growth begins at this temperature. The gene for aflatoxin biosynthesis is called aflR (Saleem Ahmad *et al.*, 2017). A study done by Bernáldez *et al.* (2017) showed that lowest concentration

aflR gene was expressed when the temperature was at 25°C when the water activity (a_w) is 0.99.

The optimum elements for *Aspergillus flavus* growth are starch, soluble sugars (sucrose and glucose), lipids, proteins and temperature at 30°C (for maximum carbon source use) (Yazid, Thanggavelu, Mahror, Selamat, & Samsudin, 2018; Giorni, Magan, Pietri, Bertuzzi, & Battilani, 2007). Yazid and colleagues (2018) have compared aflatoxicogenic *Aspergillus flavus* culture in milled media and extract media that was boiled with water and extracted by filtration using muslin cloth. The milled formulation of media had shown significantly higher concentration of *Aspergillus flavus* and AFB1 compared to the hot water extraction. This was because sugar content required by *Aspergillus flavus* growth had reduced along boiling and filtration. On top of that, Liu *et al.* (2016) stated that direct starch addition increased the growth of hyphae. Lipid is also essential for secondary metabolites production. Since lipid is insoluble in water, lipid cannot be extracted using hot water extraction (Hidalgo & Zamora, 2006). In addition to that, other factors that affect aflatoxin synthesis are carbon, nitrogen and plant metabolites (Coppock, Christian, & Jacobsen, 2018).

2.3.3 AFB1 Permissible Standard Level

AFB1 can be commonly found in grains including rice (Mardani *et al.*, 2011). Since AFB1 is a stable compound, it is hard to be destroyed even during rice processing. European Union has set a minimum tolerable limit for AFB1 as 2 µg/kg in rice while the Institute of Standards and Industrial Research of Iran (ISIRI) has established a limit of 5 µg/kg of AFB1 in rice. According to the Malaysian Food Regulations (1985), rice content must not exceed 35 ppb (for any mycological contaminant including AFB1) (Ali, 2000). The difference in the limits is because national dietary exposure varies

between developed and developing countries. Generally, mean aflatoxin in diet in developed countries are <1 ng/kg body whereas the approximate in some sub-Saharan African countries could go beyond 100 ng/kg per day (WHO 2018). Therefore, it is important to store rice at certain temperature and moisture to control aflatoxigenic *Aspergillus spp.* growth.

Previously, airborne AFB1 were recorded to have ranged from 0.4 – 7.6 ng/ m^3 in peanut mill (Sorenson *et al.* 1984) and 0.23-100 ng/m^3 in grain harvesting industry (Selim, Juchems & Popendorf, 1998). It was found that a cattle farm in Normandy, France had detectable AFB1 but the level was under lower limit of quantitation (LOQ) of 0.09 ng/filter. On daily basis, a person should not inhale more than 19 ng/kg of airborne AFB1 (Kelman *et al.*, 2004). In another contrasting finding, Hardin *et al.*, (2009) suggested that the generic airborne particles including AFB1 must not exceed 30 ng/m^3. Notwithstanding, permissible airborne limit specific for AFB1 has yet to be established.

2.4 Health Effects of Aflatoxin on Humans

2.4.1 Effects on Respiratory System

As previously been discussed, inhalation is the main route of entry for AFB1. Aflatoxin exposure have been previously reported to cause bronchopulmonary aspergillosis or fungal rhinosinusitis in humans (Chaudhary and Marr 2011; Hutcheson, Schubert, and Slavin 2010). Hence, this particular section will discuss on the effects of aflatoxin on human health. The autopsy of a textile and two agricultural workers who died of pulmonary interstitial fibrosis had revealed AFB1 in their lungs possibly from occupational exposure via inhalation (Dvořáčková and Píchová 1986). A 68-year-old

chemical engineer who worked for three months had died of pulmonary adenomatosis with AFB1 detected in his lung (DvořáčKová 1976).

The model illustrates mechanism of *Aspergillus fumigatus* killing in a host (Figure 2.3). This model applies to any spore type. First, fungal nuclei proliferate causing fungal burden (1) that triggers macrophages (2) to produce cytokines (3). The cytokines then induce recruitment of neutrophils and inflammatory dendritic cells (4). Then, neutrophils kill the fungus (5) while dendritic cells produce cytokines (6). Later, neutrophils facilitate dendritic cells efflux (7). Finally, cytokines, dendritic cells and neutrophils degrade (not shown in the diagram).

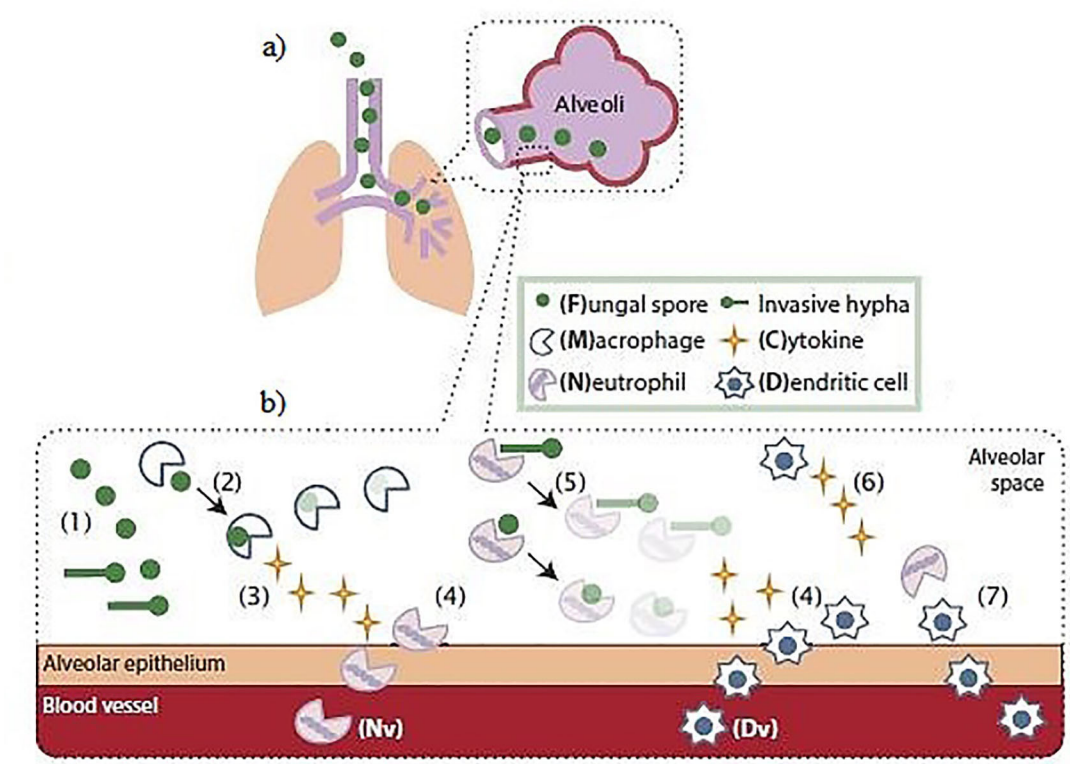

Figure 2.3 Mechanism of spore inhalation adapted from Tanaka *et al.* (2015). Illustrations of fungal exposure (b) killing for *Aspergillus fumigatus* (c) Diagram for cellular interactions

AFB1 may encourage Src phosphorylation that will eventually lead to lung cancer cell migration (Cui *et al.,* 2015). Massey *et al.* (2000) reported that AFB1 induces deoxyribonucleic acid (DNA) damage through epoxidation that results in point mutation at guanine residues (Massey *et al.*, 2000). It was shown that microsomes in rabbit lung had significantly high activity of cytochrome P450-mediated metabolism of AFB1 to a DNA-binding metabolite particularly in non-ciliated bronchiolar epithelial cells. Mulder *et al.* (2014) portrayed that nucleotide excision repair (NER) that functions to repair DNA in wild type mice increased when there was AFB1 chronic exposure. However, when p53 knockout mice was used, NER decreased which concluded that allele loss in p53 had reduced NER response. This concludes that AFB1 not only can physically damage lung cells but also mutate them if inhaled for a long period.

2.4.2 Effects on Central Nervous System

Neurons in brain cells have high metabolic rate so constant oxygen flow is required because oxygen scarcity will cause brain cells to die within minutes. Aflatoxins may inhibit nerve function by producing reactive oxygen species (ROS) like AFB-8,9-epoxides that will then form oxidative stress factors, DNA and protein adducts that will trigger cell death (Brown, Bren, Stone & Guengerich, 2009; Halliwell, 2006; Johnson *et al.*, 1997). ROS can also cause aging, degenerative disease and brain cancers (Halliwell 2006). AFB1 was treated in rats and mouse and the results show that its neurotransmitters in the animals changed. Cognitive functions, memory and learning ability in an individual may be affected as AFB1 could cause decrease in brain acetylcholinesterase enzymes (Coulombe, 2013). Ingestion of AFB1 could cause inefficient liver function making it unable to clear ammonia from the body that will

eventually lead to hyperammonemia (high ammonia content in blood). Ammonia could cross the blood-brain barrier in the brain causing brain cells cytotoxicity. Apart from that, AFB1 was linked to Reye's syndrome, a condition in children whereby neurons degenerates and cerebrum swells. This will cause symptoms like headaches, insomnia and coordination loss (Thrasher & Crawley, 2009; Butterworth, 2002).

2.4.3 Effects on Reproductive System

It was reported that animals that fed on aflatoxin-contaminated foods has disrupted reproductive system. Hormone activity, sterility and reproductive abilities in experimental animals are affected by interference of aflatoxins. Female animals exposed to aflatoxin have decrease in ovary sizes, uterine sizes and even had implantation loss. As for male animals, the sperm cells morphology distorted and the number of primary spermatocytes as well as spermatids dropped (Hasanzadeh & Rezazadeh, 2013; Fapohunda, Ezekiel, Alabi, Omole & Chioma, 2010). Teratogenic effect of aflatoxin was described in cows and rabbits whereby deformed calves was born and rabbit embryos had eye sockets and liver enlargements (Wangikar, Dwivedi, Sinha, Sharma, & Telang, 2005; Alberta Agriculture, Food & Rural Development, 2003). Additionally, infertility in men has shown to be associated with dietary aflatoxin (Gupta, 2011; Shuaib *et al.* 2010; Uriah, Ibeh & Oluwafemi, 2001; Ibeh, Uraih & Ogonar, 1994). This is because AFB1 can penetrate blood-testis barrier to disrupt spermatogenesis (Ataman *et al.,* 2014). AFM presence in breast milk is detrimental to newborns. Infants were reported to have low birth weight and jaundice risk factor associated with aflatoxins (Shuaib *et al.* 2010).

2.4.4 Effects on Immune System

Humans regularly inhale hundreds of conidia daily. Conidia inhaled by healthy hosts (under the usual circumstances), will be cleared by epithelial mucociliary defense mechanisms and conidia that managed to escape to alveoli will have to deal with the alveolar macrophages (Park & Mehrad, 2009).

One of the reasons why aflatoxin is very invasive is that it can suppress immune functions by changing T-cell phenotypes thus reducing the number of B cells in human with Human Immunodeficiency Virus (HIV). In another study done among Ghanaians, significant correlation was shown between high levels of AFB1 with low levels of CD3+CD69+ and CD19+CD69+; components in activating T and B lymphocytes in immune system (Jiang *et al.* 2005). In a study done by Peng *et al.* (2016), AFB1 ingestion could lead to lowering of T-cell subsets and oxidative stress in the thymus of broilers. Besides that, aflatoxin could also synergise with chronic hepatitis B virus infection to cause hepatocellular carcinoma (Park, Kim, Kim & Moon, 2015).

Table 2.2 shows the studies conducted on immune system suppression by AFB1 (Gong, Watson & Routledge, 2016). In a study done by Allen *et al.* (1992) showed that children with high *Plasmodium falciparum* in blood has higher mean aflatoxin albumin AF-alb (p=0.01). In addition, Keenan *et al.* (2011) discovered that participants with the highest AF-alb quartile had higher chances of tuberculosis.

Table 2.2 Immune Suppression by AFB1

Study	Location/study/design/population characteristics	Aflatoxin exposure	Immune markers	Relationship between aflatoxin exposure and child growth impairment
Allen *et al.* (1992)	- The Gambia - Cross-seectional - -391 children aged 3-8 y	- AF-alb biomarker (n= 323) - Mean (log) 4.05 pg/mg	- Malaria – *Plasmodium falciparum* parasitemia - Antibody titre – asexual stages of *P. falciparum* - -HBV – HbsAg	- Mean AF-alb was significantly higher in children with *P. falciparum* parasitemia compared with children with no *P. falciparum* parasitemia (P = 0.01). - Mean AF-alb levels were higher in children who were HbsAg positive than those who were HbsAg negative
Turner *et al.* (2003)	- The Gambia - Cross-sectional - 472 children aged 6-9 y	- AF-alb biomarker - Detected in 434/466 (93%) - GM (95% CI): 22.3 (20.3, 24.5) pg/mg	- Secretory IgA in saliva (sIgA) - Cell-mediated immunity (CMI) - Antibody responses to both rabies and pneumococcal polysaccharide vaccines	- Children with detectable levels of AF-alb had significantly lower sIgA compared to those with non-detectable AF-alb levels (P,0.0001). - 1 of the pneumococcal antibody titers was weakly associated with F-alb (P = 0.05).
Jiang *et al.* (2005)	- Ghana - Cross-sectional - 64 adults aged 19 to 86 y	- AF-alb biomarker - Mean (SD**) AF-alb: 0.997 (0.40) pmol/mg	- Leukocyte immunphenotypes - Lymphoproliferaive response of CD4+ T cells - Cytokine production by CD8+, CD4+ and CD3-, CD56+ cells - Monocyte phagocyte functions	- Participants with high levels of AF-alb, compared to those with low levels had lower percentages of CD3+CD69+ and CD19+CD69+ cells (P=0.002), and lower percentages of CD8+ T cells that contained perforin or perforin and granzyme A (P = 0.012). - AF-alb concentrations were negatively associated with CD3+CD69+ (P=0.001) and CD19+CD69+ (P = 0.0032) cells after adjustment for age and other immune parameters.

Table 2.2 Continued

Study	Location/study/design/population characteristics	Aflatoxin exposure	Immune markers	Relationship between aflatoxin exposure and child growth impairment
Jolly *et al et al.* (2011)	- Ghana - Cross-sectional - 155 HIV positive adults - 159 HIV negative adults	- AF-alb biomarker - HIV positive mean (SD) AF-alb: 1.06 (0.60) pmol/mg - HIV negative mean (SD) AF-alb: 0.91 (0.46) pmol/mg	- Viral load - CD4 count - Liver function parameters - HBV, HCV and malaria infection parameters	- HIV positive participants with high AF-alb levels (>0.93 pmol/mg albumin based on group median) showed statistically significant increased odds of having higher HIV viral loads (OR, 2.84; 95% CI, 1.17-7.78) and higher direct bilirubin levels (OR, 5.47 95% CI, 1.03-22.85) compared to the HIV+ group with lower AF-alb levels (<0.93 pmol/mg). - Higher levels of AF-alb were associated with lower levels of albumin (P=0.01) as well as higher levels of total bilirubin (P=0.01) and direct bilirubin (P=0.01) in HIV positive participants.
Keenan *et al.* (2011)	- Ghana - Cross-sectional - 141 HIV positive adults	- AF-alb biomarker - Median AF-alb: 0.94 pmol/mg	- CD4+ T-cells - Viral load - Malaria - Tuberculosis - HBV - Pneumonia	- Participants in the highest AF-alb quartile had a higher risk of tuberculosis (HR, 3.39; 95% CI, 1.15-9.98; P=0.03) compared to those in the lowest quartile.

Source: Gong, Watson & Routledge (2016). *y: year, **SD: standard deviation

2.5 Workplace AFB1 Exposure

Workplace exposure is defined as contact with a potentially harmful physical, chemical or biological agent as a result of one's work (U.S Department of Health and Human Services 2019). It is evident that aflatoxins are present in food like rice, peanuts and flour (Hassane *et al.,* 2017; Reddy, Farhana, & Salleh, 2011; Mohd Rosni, Chye, Abidin & Ayub, 2007). This signifies that food handlers are exposed to aflatoxins during handling and processing.

Food manufacturing involves multi-stage processing. For example, rice mills have drying, dehusking, polishing and storage area. Dust concentration, humidity and temperature may vary in different areas and activities. In a study done on commercial food grains, nine out of 13 rice samples was contaminated with AFB1 with mean level of 1.75 µg/kg (Reddy, Raghavender, Salleh, Reddy & Reddy, 2017). In another study done by Awad (2007) at flourmill in Egypt, suspended dust (mg/m^3) was measured. Flour packing and store recorded the highest suspended dust (7.8 $mg/m^3 \pm 5.2$) while the lowest was at the outside of the flour mill (1.15 $mg/m^3 \pm 0.47$). This implies that different working section and activities influence total dust (mg/m^3).

Aside from that, rice mill types also influence dust production. In Thailand, Batsungneon and Kulworawanichpong (2011) had measured and compared total dust produced by wooden rice mill with iron rice mill at different areas of processing. Paddy pouring station showed the highest total dust for both type of rice mill; wooden rice mill (22.44 mg/m^3) and iron rice mill (25.00 mg/m^3). The lowest amount of dust for wooden rice mill was 1.58 mg/m^3 at rice sack station while for iron rice mill, the lowest amount of lowest amount of dust was at bran filling station (5.00 mg/m^3).

In the previous studies, researchers had found dose-relationships between years of exposure on lung functions of workers. A study done in Iran had shown that flourmill workers who work for five or more years had significantly lower mean FEV1 and FVC percent when compared with controls (Moghaddasi, Mirmohammadi, Ahmad, Etemadi Nejad & Yazdani, 2013). Spirometry study carried out by Melo, Konda, Shah, and Padwale (2016) proved that spirometric values decreases showed positive correlation with exposure time.

2.5.1 AFB1 Entry Routes

AFB1 takes effect on humans as they enter human bodies. The most common entry routes are inhalation, dermal and ingestion.

Inhalation is the quickest way AFB1 can penetrate from lung into bloodstream as mycotoxin-producing spores are <1 micron in size (Brewer *et al.*, 2013). Any particles that are smaller than 10 μm can easily diffuse into bloodstreams. Figure 2.4 shows the diagram of human respiratory system. The inhaled particles can be deposited or exhaled again. There are five deposition mechanisms; sedimentation, inertial impaction, diffusion, interception and electrostatic deposition. Usually, larger particles (10 um) will be deposited in tracheobronchial airway region while smaller ones could penetrate alveolar region which is magnified in the diagram (maximum 2 μm diameter) (WHO, 1999).

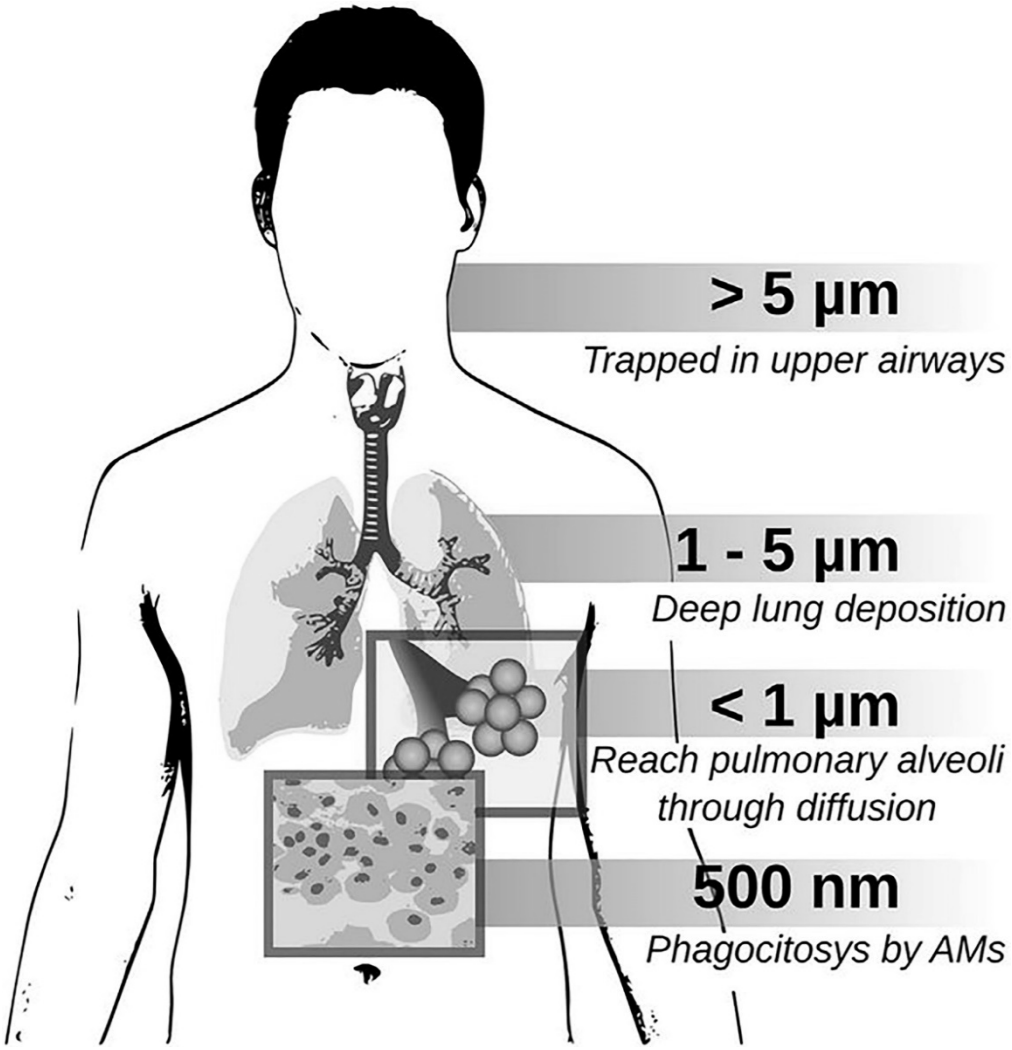

Figure 2.4 Size of particle deposition at different parts of respiratory system

Source: Costa, Pinheiro, Magalhães, Ribeiro, Seabra, Reis, & Sarmento (2016)

AFB1 is closely related to ingestion as serious cases occurrence were related to consumption of contaminated food (Bbosa *et al.*, 2013; Hamid *et al.*, 2013; Mohd-Redzwan, Jamaluddin, Abd-Mutalib, & Ahmad, 2013). The incidence of liver cancer by cereal ingestion contaminated with AFB1 was 1.5 in 100,000 adults and 2.3 in 100,000 children per year (Huong *et al.* 2016).

Aside from grains, milk is deemed as one of the main sources of aflatoxin. This is quite alarming as infants and children regularly consume milk (Boudra, Barnouin, Dragacci & Morgavi, 2007). When a breastfeeding mother ingests AFB1, it will be

metabolised by the liver and AFM1 will appear in breastmilk after 2-3 days. It also takes 2-3 days for AFM1 to disappear from breastmilk (Prandini *et al.* 2009). As for dairy product, 26% of 110 raw milk samples from milk shed in Ethiopia had AFM1 exceeded 0.05 μg/l (Gizachew *et al.* 2016). This shows that children are prone to ingest AFB1 in their diet.

Albeit data on AFB1 dermal exposure is limited, there few evidences that stated that AFB1 can penetrate through skin. An experimental study was carried out using cultured human skin to observe AFB1 permeability and it was discovered that AFB1 showed medium permeability compared to other mycotoxins with permeability rate of $8.20 \times 10{-}4$ cm/h. Historical study showed that AFB1 in methanol transferred through skin at a slow and insignificant rate (Riley, Kemppainen & Norred, 1985). Then, another study observed dermal penetration by applying AFB1 topically together with tumour promoter 12-tetradecanoyl phorbol myristate acetate (TPMA). After 13 weeks, all mice developed carcinomas (Rastogi, Dogra, Khanna & Das, 2006).

2.5.2 Safety Practice at Work

A worker from Bangladesh had severed his middle finger on the left hand when it was stuck between safety barricade and body static of trailer while transferring sacks of fertilisers (Kaur, 2019). This is one of the many incidents happened in Malaysia due to negligence of safety practice at work.

In 1994, Occupational Safety and Health Act (Act 514) was enacted to protect workers' safety, health and welfare. The act consists of 15 parts including general duties of employers and employees. Employers are responsible to provide and maintain hazard-free work environment. On the other hand, employees should cooperate with employer and observe safety of himself and others. Despite that, safety practice at work

are still overlooked. For instance, a) wearing suitable PPE b) good housekeeping practice; c) good hygiene practice.

Hazards exist in many forms, so it is always essential for workers to protect themselves. In pursuance of that, workers should wear suitable PPE at workplace where hazards are present. PPE and hazard types are summarised in Table 2.3 (Health and Safety Executive, 2013).

Table 2.3 PPE and Hazard Types

Protection	PPE Type	Hazards
Eyes	Safety spectacles, goggles, face shields.	Chemical/metal splash, dust, gases, radiation.
Head	Helmets, hard hats, bump caps.	Falling/flying objects, bumping, entanglement of hair
Lung	Respirator, disposable mask, breathing apparatus	Dust, vapour, gas, low-oxygen environment
Eyes	Safety spectacles, goggles, face shields.	Chemical/metal splash, dust, gases, radiation.
Head	Helmets, hard hats, bump caps.	Falling/flying objects, bumping, entanglement of hair

Source: Health & Safety Executive (2013)

Awareness on the importance of PPE is still low. Supporting this, rice mill workers in Hyderabad do not wear PPE because PPEs were not available and they were unaware of it (Siddiqui, Akhund, Memon, Khoso & Imad, 2018). Similarly, Nepali welders who are aware of health hazards are twice more likely to use PPE than those who are unaware (Budhathoki, Singh, Sagtani, Niraula & Pokharel, 2014). On another note, workers refuse to wear PPEs because PPEs are uncomfortable or impede movements (Joko, Dewanti & Dangiran, 2020; El Hosseini, Ghanem & Gamal, 2019; Conroy, Klene, Koppa & Park; 2017). Consequently, workers who do not wear PPE such as mask have respiratory symptoms and reduced lung function (Girma & Kebede,