UNIVERSITI PUTRA MALAYSIA

BIOLOGICAL RESPONSES OF INHALING GAS AND PARTICULATE MATTER FROM BIOMASS BURNING ON THE RESPIRATORY SYSTEM OF RATS

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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DEDICATION

This thesis is dedicated to:

My wife, Astri Fitriani
My sons Dzaki Muhammad Iffanda and
Magistra Hazmi Ichsani

Your constant encouragement, sacrifice and support is highly appreciated

Late father, Saiban Dwijowinarto
My mother, Suparni
Father in law, Asmuin
My mother in law, Sulastri

Your "do'a" for my success is very much acknowledged

Brothers and Sisters

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Trans-boundary air pollution from biomass burning like forest fire is a recurrent environmental problem in Southeast Asia. This raises inevitable issues of the impact of increasing air pollution on human health. Biomass burning releases large amounts of particulates (solid carbon combustion particulates) and gases. Uncontrolled forest fire in Southeast Asia especially Indonesia and Malaysia has caused smoke pollution (haze). The haze episodes in Southeast Asia contributed to substantial health problem of the public in 1997 and early 1998. Exposure to gas and particulate air pollution resulted in adverse health effects i.e. an increase in the morbidity and mortality due to respiratory and cardiovascular diseases however the biological mechanisms responsible for this association are not clear. This study was carried out to determine the effects of particulate matter and gases from biomass burning on the respiratory system using experimental animals in a laboratory simulation. *Spraque-Dawley* rats were used in this experiment. The experimental rats were exposed to the smoke from biomass burning for 2 hours per day for six days/week in a chamber and injected with 1 mg Keyhole Limpet hemocyanin (KLH) in 0.2 ml solution intravenously on day 0 and 8. Control animals were sham- injected with sterile deionized water and introduced into the chamber but not exposed to pollutants. Every 2 weeks, 6 rats from each group were sacrificed and airway tissue, blood and serum were collected for hematological, histological, and immunological analysis. The histological parameters studied include the number of goblet cells, the mucus gland size and the size of alveolus as examined under light microscope and by analysis of the mean average number and width of surface area.

Result of the air quality showed that the concentration of particulate matter (PM_{10}, $PM_{2.5}$ and PM_1) in the smoke was very high compared to the control. The average of PM_{10}, $PM_{2.5}$ and PM_1 in the smoke were 2414.45±190.63 $\mu g/m^3$, 379.46±20.01 $\mu g/m^3$, and 201.30±18.95 $\mu g/m^3$. The biomass burning also produced gas pollutants such as CO, NO_2, H_2S, O_3, NH_3, and SO_2. However, only concentration of CO and NH_3 showed significant differences compared with the control. The histology study indicated that the number of goblet cells of treatment group

increased during the study. The increase in the size of mucus gland and alveolar size was in accordance with the period of exposure. Likewise, the increase in concentration of mucus was parallel to exposure period. Level of Immunoglobulin G (IgG) type from ELISA analysis showed significant difference between treatment and control groups. Exposure of biomass smoke also gave significant effect in the number of hematocrite value, WBC and alveolar macrophage number. The results suggest that exposure to gas and particulate matters from biomass burning would badly affect the respiratory system.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

TINDAK BALAS BIOLOGI KESAN MENGHIDU GAS DAN BAHAN TERAMPAI DARIPADA PEMBAKARAN BIOJISIM KE ATAS SISTEM RESPIRASI TIKUS

By

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Pengerusi: Professor Madya Hamdan Noor, Ph.D.

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Pencemaran udara merentasi sempadan akibat daripada pembakaran biojisim seperti pembakaran hutan adalah merupakan masalah persekitaran terbaru yang berlaku di Asia Tenggara. Pembakaran biojism menghasilkan partikel dan gas dalam jumlah yang banyak. Pembakaran hutan yang tidak terkawal di Asia Tenggara khasnya di Indonesia dan Malaysia menyebabkan pencemaran asap (jerebu). Episod jerebu di Asia Tenggara adalah merupakan antara penyebab penting kepada gangguan/risiko kesihatan masyarakat pada tahun 1997 dan awal 1998. Pendedahan gas dan partikel terampai memberikan kesan yang membahayakan kesihatan iaitu menyebabkan meningkatnya angka kesakitan dan kematian pada penyakit salur pernafasan dan kardiovaskular tetapi kaitan mekanisme biologi di antaranya belum jelas. Kajian ini dijalankan bagi menentukan kesan bahan terampai dan gas-gas daripada pembakaran biojisim ke atas sistem respirasi haiwan kajian

dengan menggunakan kaedah simulasi makmal. Tikus *Spraque-Dawley* digunakan di dalam kajian ini. Tikus kajian didedahkan kepada asap pembakaran biojisim selama 2 jam sehari selama 6 hari/minggu di dalam satu *chamber.* Tikus rawatan disuntik dengan I mg Keyhole Limpet hemocyanin (KLH) dalam 0.2 ml larutan melalui intravena pada hari 0 dan hari ke-8. Haiwan kawalan disuntik dengan air steril nyahion dan dimasukkan ke dalam *chamber* tetapi tidak didedahkan kepada bahan pencemar. Setiap 2 minggu, 6 ekor tikus dibunuh dan tisu organ respirasi, darah dan serum diambil bagi analisis hematologi, histologi dan immunologi. Parameter histologi yang dikaji termasuklah bilangan sel goblet, saiz kelenjar mukus dan saiz alveolus yang diperiksa menerusi mikroskop cahaya dan juga analisis bilangan purata dan keluasan permukaan.

Keputusan kualiti udara menunjukkan kepekatan bahan terampai (PM_{10}, $PM_{2.5}$ dan PM_1) dalam asap adalah sangat tinggi berbanding kawalan. Purata nilai PM_{10}, $PM_{2.5}$ dan PM_1 daripada asap di dalam *chamber* adalah 2114.45 ± 190.63 $\mu g/m^3$, 379.46 ± 20.01 $\mu g/m^3$ dan 201.30 ± 18.95 $\mu g/m^3$. Pembakaran biojisim juga menghasilkan gas-gas pencemar seperti CO, NO_2, H_2S, O_3, NH_3 dan SO_2. Tetapi hanya kepekatan CO dan NH_3 menunjukkan perbezaan signifikan berbanding dengan kawalan. Kajian histologi menunjukkan bilangan sel goblet kumpulan rawatan didapati meningkat semasa kajian. Pertambahan saiz alveolus dan kelenjar adalah bersesuaian dengan tempoh pendedahan. Peningkatan kepekatan mukus juga selari dengan tempoh pendedahan. Aras *Immunoglobulin G* (IgG) daripada analisis *ELISA* menunjukkan perbezaan signifikan antara rawatan dan kawalan. Kajian ini juga memberikan kesan terhadap peningkatan jumlah sel darah merah dan alveolar makropage. Keputusan ini mencadangkan bahawa pendedahan kepada gas dan bahan terampai daripada pembakaran biojisim boleh memberi kesan buruk ke atas sistem respirasi.

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AM	: Alveolar Macrophage
BAL	: bronchoalveolar lavage
BM	: Bone marrow
Ca	: Calcium
COH	: Coefficient of haze
COPD	: Chronic obstructive pulmonary disease
DEP	: Diesel exhaust particles
ELISA	: Enzym-Linked Immunosorbent Assay
EPA	: Environmental Protection Agency
FEV1	: Forced expiratory volume in 1 second
FP	: Fine particles (usually PM2.5 or PM2.1)
FVC	: Forced vital capacity
H_2SO_4	: Sulphuric acid
HNO_3	: Nitric acid
IgG	: Immunoglobuline G
Il-6	: Interleukin-6 cytokine
Il-8	: Interleukin-8 cytokine
KLH	: Keyhole Limpet Hemocyanin
MAQG	: Malaysian Air Quality Guideline
NAAQS	: National Ambient Air Quality Standards
NaCl	: Sodium chloride
NH_3	: Ammonia

NO_2	: Nitrogen Dioxide
O_3	: Ozone
PAHs	: Polycyclic aromatic hydrocarbons
PBS	: Phosphate buffered saline
PM	: Particulate matter
PM_1	: Particulate matter less than or equal to 1 micrometers
PM_{10}	: Particulate matter less than or equal to 10 micrometers
$PM_{2.5}$	: Particulate matter less than or equal to 2.6 micrometers
PMN	: Polymorphonuclear leukocyte
Ppm	: Parts per million
RBC	: Red Blood Cells
RS-	: Negative rat control serum
RS+	: Positive rat control serum
SO_2	: Sulphur Dioxide
SO_4	: Sulphate ion
TB	: Tracheobronchial
TNF	:Tumour necrosis factor (cytokine)
TPM	: Total Particulate Matter
TSP	:Total suspended particulate
$\mu g/m^3$	: Microgram per cubic meter
µm	: Microns
WBC	: White blood Cells

CHAPTER 1

INTRODUCTION

Global biomass burning is an increasingly important issue occurring on Earth today due to the possible short and long term atmospheric damages. Biomass burning results in the release of various gases into the atmosphere which either contribute directly to the greenhouse effect and global warming or act as intermediate reactants that interact with other gases to harm the atmosphere. Atmospheric gases are not solely produced by biomass burning—fossil fuel burning, application of fertilizers, the increased size of rice fields and cattle production, as well as manmade chemical production all contribute to the increase of harmful gases into the atmosphere [Levine, 1998a]. All these forms, including biomass burning, are the result of human industry and technology, which directly impact the environment and the future of the planet. The increase of gases leads to global warming, climate change, and other environmental changes that need to be controlled to ensure the habitability of the planet. Biomass burning takes on many different forms and has different causes, but this case primarily focuses on forest fires, specifically those in Southeast Asia, a large region that is being increasingly studied for its emission contributions and effects on the atmosphere.

On an annual basis, estimates indicate that biomass burning may lead to the chemical production of approximately 38 percent of the ozone in the troposphere.

The burning also produces about 32 percent of the CO_2 emissions in the world, 39 percent of particulate organic C, and more than 20 percent of the H_2, nonmethane hydrocarbons [NMHC], CH_3Cl, NO_2 and Particulate matter [Levine, 1998a]. There are six different types of biomass burning that result from agricultural and human activities: [1] the clearing of forest and brush land for agricultural use, [2] the control of brush and other accumulations on grazing and crop lands, [3] the nutrient regeneration in grazing and crop lands, [4] the control of fuel accumulation in forests, [5] production of charcoal for industrial and domestic uses, and [6] the energy production for cooking and heating. Wildfires, which can also be naturally induced, are the other major type of biomass burning that can be induced by human activity [Levine, 1998b and 1998c].

In 1997, uncontrolled forest fires burning in the Indonesian states of Kalimantan and Sumatra, combined with severe regional drought, depressed mixing heights and prevailing winds, resulted in a regional air pollution episode of biomass smoke which impacted Indonesia, Malaysia, Singapore, Southern Thailand, Brunei and Southern Philippines. In particular, several large urban areas such as Singapore, Kuala Lumpur and Kuching were affected. Biomass smoke pollution from the forest fires resulted in an elevated level of particulate air pollution for a period of approximately 2 months in many areas [beginning in late July], with the most severe episode occurring during the month of September. During the episode a State of Emergency was declared in Sarawak, Malaysia as 24-hr PM_{10} reached as high as 930 $\mu g/m^3$, which was more than 15 times higher than the normal level [Braure, 1998]. In Malaysia, only few people were reported to have died from respiratory problem as a result of the fire, but the true long term health effects of the millions of people living in the areas worst affected will not be ascertained for many years to come. Health experts stated that breathing the air in the worst affected areas of the smoke haze was equivalent to smoking up to 80 cigarettes a day [Abbey *et al.*, 1998]. American Lung Association [1998] recorded a two to three fold increase in the number of respiratory diseases when particle concentrations reached up to 450 $\mu g/m^3$ of PM_{10}. In Singapore, hospital attendances increased around 30 percent while particle concentrations dose three to four folds to values between 100 and 150 $\mu g/m^3$ PM_{10} during September and October 1997 [Anonymous, 1998a]. In southern Thailand, a 7 to 8 percent increase of respiratory visits and admissions was attributed to the haze occurrence in late September and early October of 1997 with a daily maximum value of 218 $\mu g/m^3$ PM_{10} [Phonboon *et al.*, 1998]. During the haze episode 1997, in Indonesia, particle pollution levels over of 2000 to 3000 $\mu g/m^3$ PM_{10} impacted the Indonesian population in many regions of Kalimantan and Sumatra. A rough estimate of economic value of the damage caused the 1997 fire and haze gives a number of 1 billion US$ for haze related damage such as short term health, lost of life, tourism, and accident, reduced crop productivity for Indonesia only [Anonymous, 1998a; Heil, 1998b].

Levine [1998b] estimated that the 1997 fires in Kalimantan and Sumatra produced emissions of 85 to 316 million tons of carbon dioxide, 7 to 52 million tons of carbon monoxide, 4 to 16 tons of particulate matter, 2 to 12 million tons of ozone, 0.1 to 4 tons of ammonia and 0.2 to 1.5 million tons of oxides of nitrogen. The lower limit of the estimate assumed that solely forest and no peat burned, while the upper limit assumed that peat fires contributed to 30 percent of the area burnt.

Particulate matter [PM] is the generic term applied to a broad class of chemically and physically diverse substances that exist as discrete particles [liquid droplets or solids] over a range of particle sizes. In contrast to the other criteria of pollutants such as carbon monoxide, PM is chemically heterogeneous. Particles are present everywhere, but high concentrations and/or specific types of particles have been found to pose serious danger to human health. Particulate pollution comes from such diverse sources like factory and utility smokestacks, vehicle exhaust, wood burning, mining, and construction and agriculture activities [Anonymous, 1998a; McClellan & Miller, 1997].

Increased concentrations of fine particles in the ambient air are associated with substantial health impacts such as acutely and chronically decreased lung function, upper respiratory infections, asthma, bronchitis, cardio-vascular diseases and increase of daily mortality. Health effect studies could not identify any clear no-effect threshold. The US-EPA defines the air quality standard for particulate matter smaller than 10-μm diameters [PM_{10}] at 150 μg/m^3 and for fine particles or $PM_{2.5}$ [diameter smaller than 2.5 μm] at 65 μg/m^3 [daily average]. These are thresholds at which public health effects are likely to be small but not