UNIVERSITI PUTRA MALAYSIA

SHORT-TERM HEALTH EFFECTS OF COPPER, LEAD ANDFIRE SMOKE ON SPRAGUE-DAWLEY RATS

MAGESWARY NAVAPPAN

FPSK(M) 2006 5

SHORT-TERM HEALTH EFFECTS OF COPPER, LEAD AND FIRE SMOKE ON SPRAGUE-DAWLEY RATS

By

MAGESWARY NAVAPPAN

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

June 2006

To my father, mother, brothers, niece, nephew and
lecturers of the Faculty of Medicine and Health Sciences,
for their understanding, patience and support
and
To all my fellow students, past and present,
who have provided the vast reservoir of
knowledge from which the content of this thesis
was made possible

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

SHORT-TERM HEALTH EFFECTS OF COPPER, LEAD AND FIRE SMOKE ON SPRAGUE-DAWLEY RATS

By

MAGESWARY NAVAPPAN

June 2006

Chairman: **Associate Professor Patimah Ismail, PhD**

Faculty: **Medicine and Health Sciences**

The toxicological effects of copper, Cu, lead, Pb, and fire smoke were determined comparatively in this study. Cu and Pb were selected due to their abundance in ambient air bound to conjugates of PM10 (US EPA, 1986). Cu is released to the atmosphere in association with particulate matter (Dameron and Howe, 1998; ATSDR, 2004). The level of Pb in the air is considerably high in the urban region especially due to automobile emission and industrial wastes. Smoke from open burning contains particulate matters that conjugate with Pb emitted from the automobile fires. Contamination by Pb and Cu, besides a few other metals, appears to be significant in water run-off from these types of fires (Lonnermark and Blomquist, 2005).

Attempts were made at determining the effects of these three types of environmental pollutants, namely, copper, lead and fire smoke on three independent groups of Sprague-Dawley rats. Thirty-two 11-week-old rats (16

male and 16 female) were allocated each for copper and lead treatment groups. Fifty rats (25 male and 25 female) were allocated for the fire smoke exposed group. The copper and lead administered groups were subdivided into 4 different concentrations, being 0 parts per million (ppm), 200 ppm, 350 ppm and 500 ppm respectively. Copper was administered in the form of copper(II) sulphate solution while lead in the form of lead acetate solution, both orally in fixed volumes of 250 mL and *ad libitum* for 4 consecutive days during which all rats were closely monitored for macroscopic lesions and behavioural abnormalities.

The rats were anaesthetised for cardiac puncture via which adequate volume of blood was collected for serum chemistry to determine levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), cholesterol, albumin, triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL), blood urea nitrogen (BUN) and total protein and to prepare thin blood smears for differential white blood cell (WBC) counts. The liver and kidney specimens were subjected to macrosopic and microscopic examinations.

The modes of exposure for the fire smoke treated rats was via inhalation and ingestion of the particulate matters found in the smoke that sediments into the drinking water or feed of the rats. Smoke was induced in a microenvironment (with a volume of 2.16 m^3) in which the rats were exposed

for 4 consecutive days. These rats and their relevant specimens were also subjected to similar macroscopic and microscopic examinations.

Basically, the concentrations of the selected serum parameters in all control rats were not observed to be within the reference range. In the treated groups (all three categories), a common dose-response relationship could not be generated from the readings obtained. No significant difference was observed statistically (based on ANOVA) among the readings of serum parameters in rats given 200 ppm, 350 ppm and 500 ppm of copper(II) sulphate to that of the control rats except the mean of ALP concentration. It was significantly different from the control group at $p< 0.05$. The lead acetate treated rats showed significant differences among the groups given 200 ppm, 350 ppm and 500 ppm in their levels of ALT, AST, ALP, cholesterol and HDL with p-values of 0.013, 0.009, 0.034, 0.002 and 0.003 respectively (The mean difference is significant at $p<0.05$.) There was not any detectable amount of copper and lead in the samples of fire smoke including water samples that were directly exposed to the smoke. The fire smoke exposed rats showed significant differences in AST, ALP, albumin and triglyceride HDL with p-values of 0.003, 0.006, 0.006 and 0.000 respectively (the mean difference is significant at $p<0.05$). None of the rats from the treated or exposed groups showed abnormal WBC count when compared to the reference range. There was no statistical significance either when compared to the control group of rats at $p<0.05$. No marked macroscopic or microscopic lesion was observed

in almost 96% of the rats experimented with their liver and kidney specimens. The cholesterol level was significantly increased in all rats including that of the control rats. Conclusions are made that a short-term exposure of 4 days did not induce any significant toxicokinetics that could affect the health of the treated rats. An association between the toxicity of copper, lead and fire smoke cannot be made either because there was no detectable amount of copper or lead in the fire smoke.

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

KESAN-KESAN KE ATAS KESIHATAN AKIBAT PENDEDAHAN JANGKA MASA SINGKAT TERHADAP KUPRUM, PLUMBUM DAN ASAP KE ATAS TIKUS SPRAGUE-DAWLEY

Oleh

MAGESWARY NAVAPPAN

Jun 2006

Pengerusi: **Profesor Madya Patimah Ismail, PhD**

Fakulti: **Perubatan dan Sains Kesihatan**

Kesan-kesan toksikologi kuprum, Cu, plumbum, Pb, dan asap yang berpunca daripada kebakaran dikaji secara komparatif dalam penyelidikan ini. Cu dan Pb dipilih disebabkan kewujudannya yang ketara di udara dalam bentuk konjugat-konjugat PM10 (US EPA, 1986). Pembebasan Cu ke dalam udara dikaitkan dengan kewujudan partikel-partikel yang sedia mengikatkannya di udara (Dameron dan Howe, 1998; ATSDR, 2004). Kepekatan Pb dalam udara juga tinggi khususnya di kawasan bandar disebabkan penghasilannya daripada eksos kenderaan bermotor serta bahan buangan industri. Pembakaran secara terbuka juga merupakan suatu sumber bagi partikel-partikel asap yang mengikat Pb yang dikeluarkan bersama asap eksos kenderaan. Pencemaran oleh Cu dan Pb, di samping logam-logam lain, adalah ketara dalam sampel-sampel air yang dialirkan di atas kawasan-kawasan yang dicemari oleh asap yang berpunca daripada kebakaran (Lonnermark dan Blomquist, 2005).

Penyelidikan untuk menentukan kesan-kesan akibat pendedahan terhadap tiga jenis pencemar alam sekitar termasuk kuprum, plumbum dan asap yang berpunca daripada kebakaran telah dilakukan ke atas tiga kumpulan tikus *Sprague-Dawley* secara berasingan.

Kajian ini melibatkan 32 ekor tikus (16 jantan dan 16 betina) dalam lingkungan usia 11 minggu untuk setiap kumpulan yang melibatkan pendedahan terhadap kuprum dan plumbum masing-masing. Manakala, kumpulan ketiga yang didedahkan kepada asap merangkumi 50 ekor kesemuanya (25 jantan dan 25 betina). Kuprum dan plumbum diadministrasi dalam 4 kepekatan yang berlainan iaitu 0 bahagian per juta (ppm), 200 ppm, 350 ppm dan 500 ppm. Sebab utama bagi pemilihan konsentrasi sebegitu adalah untuk memperoleh suatu takat yang mampu memberi kesan kepada tikus tanpa membunuhnya. Kuprum diberi kepada tikus dalam bentuk larutan kuprum(II) sulfat manakala plumbum dalam bentuk larutan plumbum asetat secara oral dalam isipadu tetap sebanyak 250 mL *ad libitum* selama 4 hari berturut-turut. Tikus-tikus ini diberi perhatian rapi di sepanjang tempoh administrasi untuk penentuan lesi makroskopik dan kelakuan yang abnormal.

Tikus-tikus dibius untuk prosedur *cardiac puncture* melalui mana darah yang secukupnya diambil untuk menjalani ujian-ujian kimia serum termasuk

penentuan kepekatan alanina aminotransferase (ALT), aspartat aminotransferase (AST), alkalin fosfatase (ALP), kolesterol, albumin, trigliserida, lipoprotein densiti tinggi (HDL), lipoprotein densiti rendah (LDL), urea nitrogen darah (BUN) and protein keseluruhan (*total protein*). Darah juga diambil bagi penyediaan slaid-slaid darah untuk pengiraan sel-sel darah putih. Spesimen-spesimen hati dan ginjal diperiksa secara makroscopik dan mikroskopik.

Mod-mod pendedahan asap ke atas tikus dalam kajian ini adalah melalui inhalasi and ingesi partikel-partikel asap yang termendak ke dalam air minuman atau makanan tikus. Asap diwujudkan dalam suatu persekitaran tersendiri (*microenvironment yang* berisipadu 2.16 m^3) di mana tikus-tikus yang dikaji mengalami pendedahan selama 4 hari berturut-turut. Ujian-ujian yang dilakukan untuk dua kumpulan yang sebelum ini juga dilakukan terhadap tikus-tikus dalam kumpulan ini.

Secara amnya, kepekatan-kepekatan parameter serum yang diperolehi daripada kumpulan kawalan tidak terlingkung dalam julat normal. Untuk tikus-tikus yang didedahkan kepada Cu, Pb dan asap pula, tiada hubungan dos-respons yang ketara. Didapati tiada perbezaan yang bererti antara bacaan-bacaan parameter serum secara statistik (berasaskan ANOVA) yang diperoleh daripada tikus-tikus yang diberi larutan kuprum(II) sulfat dalam kepekatan 200 ppm, 350 ppm dan 500 ppm kecuali kepekatan ALP. Ia

mempunyai perbezaan yang bererti pada $p<0.05$. Kumpulan tikus yang diberi larutan plumbum asetat menunjukkan perbezaan yang bererti bagi nilai-nilai kepekatan ALT, AST, ALP, kolesterol dan HDL dengan nilai-nilai p sebanyak 0.013, 0.009, 0.034, 0.002 dan 0.003 masing-masing jika dibandingkan di antara kumpulan-kumpulan yang dikaji dengan kawalan. (Sisihan min adalah bererti pada tahap $p<0.05$.) Didapati tiada Cu dan Pb yang wujud dalam sampel asap dan air yang dikaji. Kumpulan yang terdedah kepada asap pula menunjukkan perbezaan yang bermakna bagi nilai-nilai kepekatan AST, ALP, albumin dan trigliserida dengan nilai-nilai p sebanyak 0.003, 0.006, 0.006 and 0.000 masing-masing. Keabnormalan dalam pengiraan sel-sel darah putih, lesi makroskopik dan lesi mikroskopik bagi spesimen-spesimen hati dan ginjal tidak kelihatan sama sekali bagi ketiga-tiga kumpulan dalam kajian ini. Kesimpulannya, pendedahan jangka masa singkat selama 4 hari tidak dapat mencetuskan sebarang mekanisme toksikokinetik yang memberi kesan terhadap kesihatan tikus-tikus dalam kajian ini. Kesan toksik Cu, Pb dan asap tidak dapat dihubungkaitkan sama sekali disebabkan ketidakwujudan Cu and Pb dalam asap.

ACKNOWLEDGEMENTS

To my beloved Chairman of the Supervisory Committee, Assoc. Prof. Dr. Patimah Ismail, thank you so much for your guidance and support throughout my being your postgraduate student. I could not have done better without your kindness both as a lecturer and as a cheerful friend all the way. Thank you again, Dr. Pat. I would also like to express my heartfelt gratitude to my co-supervisors, Assoc. Prof. Dr. Sabariah Abdul Rahman, the Deputy Dean of Faculty of Medicine and Health Sciences and Assoc. Prof. Dr. Muhammad Nazrul Hakim Abdullah, the Head of Pharmacology and Toxicology Unit, for their endless guidance and support throughout my study. I would also like to take this opportunity to say that I truly feel honoured and lucky to have been assigned to such jovial, charming and helpful supervisors during my duration of study.

I also wish to express my heartfelt gratitude to Prof. Dr. Mohd. Zamri Saad and Mr. Tuan Haji from the Histology Department, and Dr. Helmi and Mr. Murthy from the Cytology Department for their invaluable technical support in the laboratories that aided in the success of this study. I also thank my wonderful fellow students, Ms. Norazila Kassim Shaari, Ms. Wong Fei Ling and Mr. Cheah Yoke Kqueen who have been extremely generous in spending their precious time handling the rats during the dissection sessions. Thanks to Mr. Yakob Nawawi, for his endless support in the animal house throughout my study.

I am also very grateful to Mr. V. Sam, Mrs. Mageswari, Mr. Elan, Mr. Rajkumar and Mr. Anba for their invaluable guidance in the electronic aspects of the production of this thesis. Last but not least, thanks to my beloved family, lecturers, friends and staffs of the Department of Biomedical Sciences for their kindness, tolerance and support in making this study a great success. I really appreciate the presence of all of you in my life.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES / PLATES

Figure **Page**

Plate

LIST OF ABBREVIATIONS

µ	micron
µm	micrometer
µmol/L	micromole per litre
AAS	atomic absorption spectrophotometry
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ANOVA	Analysis of Variance
API	Air Pollution Index
AST	aspartate aminotransferase
ATSDR	Agency for Toxic Substances and Disease Registry
BUN	blood urea nitrogen
bw	body weight
CNS	central nervous system
CO	carbon monoxide
Cu	copper
DNA	deoxyribonucleic acid
ENSO	El Nino Southern Oscillation
g	gram
G	gauge
g/dL	gram per decilitre
GFAAS	granite furnace atomic absorption spectrophotometry

GFR	glomerular filtration rate
GST	glutathione S-transferase
H & E	haematoxylin and eosin
HDL	high density lipoprotein
IPCS	International Programme on Chemical Safety
IU/L	international unit per litre
LD	lethal dose
LDL	low density lipoprotein
LEC	Long-Evans Cinnamon
LOAEL	low observed adverse effect level
MAQI	Malaysian Air Quality Index
mg	milligram
mg/kg	milligram per kilogram
mg/L	milligram per litre
mL	millilitre
mm	millimetre
NOEL	no observed effect level
O_2	oxygen
p	probability
Pb	lead
PM	particulate matter
PCE	polychromatic erythrocytes

ppm	parts per million
PSI	Pollution Standards Index
RBC	red blood cell
SD	standard deviation
SEA	South East Asia
SGOT	serum glutamic oxaloacetic transaminase
SOD	super oxide dismutase
SPM	suspended particulate matter
TG	triglyceride
TSP	total suspended particulate
UNPEX	Uniform International Pollutants Index
US EPA	United States Environmental Protection Agency
US RDA	United States Research Dietary Allowance
WBC	white blood cells
WHO	World Health Organisation

CHAPTER 1

INTRODUCTION

Malaysia has experienced drastic episodes of environmental pollutions; be it land, water or air pollution. The general air quality of Malaysia since 1970 has deteriorated (Awang *et al.*, 2000). The air pollution comes mainly from land transportation, industrial emissions and open burning sources. The undeniable fact is that land transportation contributes the most to air pollution. The results of the monitoring of air quality with relation to its pollutants indicated that suspended particulate matter (SPM) was the predominant pollutant besides nitrogen dioxide, NO_2.. In Malaysia, other pollutants such as lead (Pb), carbon monoxide (CO) and sulphur dioxide (SO_2) are also observed in several big cities (Afroz *et al.*, 2003).

Copper (Cu) is released to the atmosphere in association with particulate matter (Dameron and Howe, 1998). The particulate matters have copper bound within them (Agency For Toxic Substances And Disease Registry, 2004). Copper basically occurs in the environment by means of conjugates bound to dust particles in the form of particulate matters or may be embedded in minerals and are generally non-bioavailable, that is, not readily decomposed by bacteria (Agency For Toxic Substances And Disease Registry, 2004). Copper is released into the environment from natural sources like forest fires, windblown dusts, volcanoes and decaying vegetation. It is also