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ASSESSMENT OF MICRONUCLEUS FREQUENCY AND RESPIRATORY HEALTH SYMPTOMS AMONG TRAFFIC POLICEMEN EXPOSED TO BTEX AND $PM_{2.5}$ IN KLANG VALLEY, MALAYSIA

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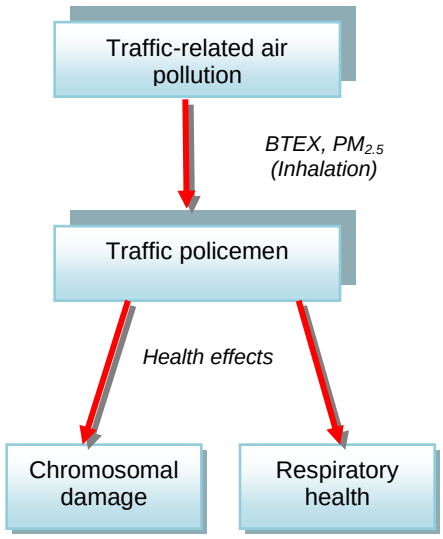
Graphical abstract



Abstract

Volatile organic compounds such as benzene, toluene, ethylbenzene, xylene (BTEX), and particulate matter (PM) with a diameter of less than 2.5 microns ($PM_{2.5}$) are often associated with traffic-related air pollution (TRAP) and harm the health of the community. This study aimed to evaluate the personal air pollutant exposure, micronucleus (MN) frequency, and respiratory health symptoms among 160 traffic policemen and 149 office workers in Klang Valley. Personal exposure concentrations for BTEX and $PM_{2.5}$ among traffic police were 390.12 μg/m³ and 140.00 μg/m³ respectively, whereas 97.64 μg/m³ (BTEX) and 23.00 μg/m³ ($PM_{2.5}$) among office workers. Statistical analysis for MN frequency between traffic policemen (6.2±2.6) and office workers (3.0±2.0) shows a significant difference ($p < 0.001$). The Chi-Square test for respiratory health symptoms indicates that only cough shows the significant differences between traffic policemen and office workers ($\chi^2 = 5.645$, $p = 0.018$, PR = 1.800). In short, this study showed that TRAP exposure would increase the chromosomal damage that can cause high MN frequency among traffic policemen and would increase the prevalence of respiratory health symptoms among urban workers.

Keywords: Volatile organic compounds, particulate matter, traffic-related air pollution, traffic policemen, chromosomal damage

Abstrak

Sebatian organik meruap seperti benzena, toluena, etilbenzena, xylena (BTEX) dan zarah terampai dengan diameter kurang daripada 2.5 mikron ($PM_{2.5}$) adalah bahan pencemar yang sering dikaitkan dengan pencemaran udara trafik serta membahayakan kesihatan komuniti. Kajian ini bertujuan untuk menilai pendedahan pencemaran udara, mikronukleus (MN) dan gejala-gejala kesihatan pernafasan di kalangan 160 anggota polis trafik dan 149 pekerja pejabat di Lembah Klang. Kepekatan pendedahan kepada BTEX dan $PM_{2.5}$ di kalangan polis trafik ialah 390.12 μg/m³ dan 140 μg/m³ masing-masing dan 97.64 μg/m³ (BTEX) dan 23.00 μg/m³ ($PM_{2.5}$) di kalangan pekerja pejabat. Analisis statistik untuk kekerapan MN antara anggota polis trafik (6.2 ± 2.6) dan pekerja pejabat (3.0 ± 2.0) menunjukkan perbezaan yang ketara (p <0.001). Ujian Chi-Square untuk gejala-gejala kesihatan pernafasan menunjukkan bahawa hanya gejala batuk menunjukkan perbezaan yang signifikan antara anggota polis trafik dan pekerja pejabat (x^2 = 5.645, p = 0.018, PR = 1.800). Secara keseluruhannya, kajian ini menunjukkan bahawa pendedahan pencemaran udara trafik akan meningkatkan kerosakan kromosom yang boleh menyebabkan peningkatan frekuensi MN di kalangan anggota polis trafik dan akan meningkatkan simptom kesihatan pernafasan di kalangan pekerja bandar.

Kata kunci: Sebatian organik meruap, zarah terampai, pencemaran udara trafik, polis trafik, kerosakan kromosom

1.0 INTRODUCTION

Urban air consists of a mixture of pollutants from different sources such as mobile sources, stationary sources, and open burning sources [1, 2]. Pollutants from traffic emission or known as TRAP were the major contributor to air pollution in urban areas [3, 4]. The urban workers such as traffic policemen are susceptible to higher exposure of TRAP. Traffic policemen are those who exposed to direct traffic or serve in the traffic unit, which has the responsibility to enforce the rules of the road. Therefore, the traffic policemen are highly exposed to air pollution caused by traffic and posed to a higher risk of health effects from TRAP exposure. Besides, traffic policemen are also representative of people who are persistently exposed to high levels of pollutants from traffic pollution [5].

The volatile organic compounds (VOCs) that are produced during fuel combustion of vehicle engines contribute 70 to 75% of VOCs in the air of the urban environment [2]. Among the VOCs emitted during fossil fuel combustion are benzene, toluene, ethylbenzene and xylene (BTEX), in which these compounds are the most studied compounds by previous researchers due to health effects [6, 7] and as a marker for VOCs exposure [8]. Furthermore, benzene is categorized in group 1 carcinogen compound by the International Agency for Research on Cancer (IARC) [9]. According to earlier researchers, the toluene to benzene (T/B) ratio in the range of 1.5 to 4.3 may indicate that BTEX or VOC sources are mainly derived from vehicle emission [10, 11, 12]. Meanwhile, m,p-xylene to ethylbenzene (m,p-X/E) ratio indicates the photochemical age of the air masses with the ratio between 3.8 and 4.4 for fresh gasoline emissions [11, 13].

About 50% of $PM_{2.5}$ emission in the urban environment was originated from TRAP, mainly from vehicle exhaust [6] and have a positive relationship with diesel engine vehicles [14]. Long-term exposure to $PM_{2.5}$ has an impact on 432,000 premature deaths in Europe [15]. Furthermore, $PM_{2.5}$ tends to induce chronic diseases because of the properties of these particles that able to deposited into the alveolar of the lung and increased the risk of inflammation, oxidative stress, and blood coagulation [16, 17]. The previous study conducted by Zhao *et al*. (2015) found that exposure to a higher level of $PM_{2.5}$ can reduce pulmonary function among traffic policemen in Shanghai, China [18].

Urban air consists of multiple chemical compounds that originated from fossil fuel combustion [17] that cause deoxyribonucleic acid (DNA) damage, which is genotoxic to the organism [19]. DNA damage acts as an endpoint impact of exposure to air pollution like polycyclic aromatic hydrocarbon PAH [20]. MN frequency from the buccal cell was a susceptible method for the assessment of DNA damage following exposure to the genotoxic agent [21] and identification in predicting the risk of cancer [22]. MN frequency can assess two classes of chromosomal damage: chromosomal breakage and aneuploidy [23]. Furthermore, this method is a non-invasive method that did not require to draw blood or other body fluid from an organism [23]. Da Rosa *et al*., (2013), mentioned that exposure to a low-level carcinogenic agent (for example, benzene) may cause

chromosomal damage that increases the MN formation in a human cell [24]. Also, long-term exposure to diesel engine exhaust can increase MN frequency [25].

Klang Valley is located in the middle of Selangor and also known as a "Greater Kuala Lumpur". This area is among the fast-growing area in Malaysia due to the rapid development of industries and community activities, as well as a highly-populated. Due to this, the number of vehicles keeps increasing every year in this area and in which the Road Transport Department Malaysia (JPJ) reported that the annual increment of vehicles on the road within this area was increased 4% yearly [26]. As a result, there were increases in anthropogenic sources of air pollution in this area and affected the health of the local community. In Malaysia, studies regarding the association of environmental and epidemiological data are limited, especially a study of air pollution and health outcome [1, 27]. Hence, this study is conducted to obtain a comprehensive understanding of the TRAP effect on the Malaysian community specifically in a Klang Vally area. This study aims to investigate the level of TRAP personal exposure level, MN frequency, and respiratory health symptoms among urban workers in the Klang Valley. The pollutants that are further assessed in this study are BTEX and $PM_{2.5}$. Besides, we would also like to investigate the association between air pollutant exposures and health effects among study subjects. This study is a cross-sectional comparative study conducted between traffic policemen that working outdoors and exposed to traffic particles during the control of the traffic flow as exposed group and office workers that working indoors and dealing with the administrative task within the Klang Valley as a comparative group.

2.0 METHODOLOGY

2.1 Study Design and Population

This study is a cross-sectional comparative study conducted among 160 traffic policemen as exposed groups and 149 office workers as a comparative or control group. Both groups are working in the Klang Valley. The random sampling method was used for the selection of participants in this study. The study was conducted from August 2017 to January 2018. Only male workers with age ranged between 20 – 60 years were selected in this study. The participants must be working in the Klang Valley area for at least one year and hold the same position during that period. The participants who had radiotherapy, chemotherapy, therapeutic drug use, and exposure to X-ray diagnosis in the previous six months were excluded in this study. Only 195 respondents were selected for personal air samples, and 215 respondents were selected for the MN frequency test. This study was approved by the Ethical Committee, Universiti Putra Malaysia (JKEUPM-2017-247).

2.2 Questionnaire

A set of questionnaire was distributed to participants to obtain sociodemographic and socioeconomic information. The questions on respiratory health were adapted from a standardized questionnaire by the American Thoracic Society for respiratory disease ATS-DLD-78. The questions were categorized into ten parts that include socio-demographic, occupational information, residential Information, outdoor and indoor residential surroundings, environmental tobacco smoke (ETS), hobbies and activities, eating habits, allergies, medical and health status, and history of respiratory health.

2.3 Air Sampling

The air sampling pump was fitted to the participant's body to collect pollutants to measure exposure levels. This air pump was attached to the participants during working hours. The cyclone was placed in the breathing zone. Sidepak AM520 Personal Aerosol Monitor (TSI) was used for measuring $PM_{2.5}$. This instrument gives real-time aerosol mass readings using light-scattering principles. The light scattering principles respond linearly to the aerosol mass concentration.

Meanwhile, BTEX compounds were collected using Low Flow Personal Air Pump Sampler (Spectrex, USA) instrument with a flow rate of 0.02 L/min by attaching to the Thermal Desorption (TD), Tenax GR stainless steel tube. The Tenax GR material consists of 2, 6-Diphenyl-p-phenylene oxide with 23% graphitized carbon and the mesh size 60/80. The pump was calibrated using Air Flow Calibrator (Go Cal. Sensidyne) before and after sampling. The used tubes were capped and stored in a cool box before transferred to the laboratory for gas chromatography (GC) analysis.

2.3 Buccal Mucosal Cell Collection

The buccal mucosal cell was collected from each participant by a sterile cytology brush. Before cell collection, the participant must rinse their mouths twice with water. For collecting buccal cells samples, the brush was inserted into the subject's mouth and rubbed firmly against the inside of the subject's cheek or underneath lower or upper lips. The cells were dipped into a 1.5 ml microcentrifuge tube containing 1 mL of phosphate buffer saline (PBS) at 0.1 M and stored in the cool box before sent to the laboratory for MN assay analysis.

2.4 BTEX Analysis

The BTEX analysis method was conducted following the US EPA method TO-17 [28] with some modifications. The Tenax GR tubes were analyzed

with thermal desorption (TD) instrument (Unity, Markes) coupled with a GC Agilent 6890 N (Agilent, USA) and mass spectrometry (MS) detector Agilent 5975C (Agilent, USA). The capillary used was 60 m length, 0.32 mm i.d, 1.80 µm film thickness. The TD desorption parameter was set at desorption: 280^{0}C, desorption time: 30 min, split flow: splitless, flow path temperature: 140^{0}C, line purge: 2 min, trap purge: 3 min and sample valve at 0.5 L. For GC/MS, the volume of sample was set at 1.0 µL with inlet temperature at 200 πC. The temperature for the oven during the first 5 min was set at 36 πC and then increased to 5 πC per min until reached 250 πC for 2 min. The detector temperature was set at 250 πC. For quality control, the multiple points' calibration was performed to create the calibration curve for BTEX. Gas standard 10 ppm (MESA, USA) was diluted proportionally with purified air in the pre-clean 10 L Tedlar bag. The limit of detection (LOD) was calculated based on three standard deviations of the lowest concentration (1 ppb). The LOD for benzene, toluene, ethylbenzene, m,p-Xylene and o-Xylene were 0.08 ppb, 0.04 ppb, 0.02 ppb, 0.02 ppb and 0.02 ppb respectively.

2.5 Micronucleus Assay

Micronucleus assay was prepared and described, according to Thomas *et al*., (2009) [29]. The microcentrifuge tubes that contained buccal cells were centrifuged for 1 min at 1000 rpm. After the centrifuge process was performed, the supernatant was discarded. By using a pulling method, the cells were smeared onto the clean slides. This cell was allowed to dry before fix with 1% of glutaraldehyde in 0.1 M phosphate buffer (pH=7.5) for 20 min. Then, the fixed cells on the slide were hydrolyzed in 5N HCl for 30 min at 27°C and washed for 5 min in distilled water. Next, the slides were stained by Feulgen reaction. The Schiff reagent was used to stain the cells for 45 min. The slides containing fix buccal cells were washed using tap water for 15 min and then counterstained with 0.1% fast green for 20 to 40 sec. After that, the slide was rinsed well with deionized water. The slides were dried for a day before analyzing the MN. The cells were examined under a light microscope (1000x) to determine the MN frequency. 2000 cells (1000 cells from each slide and for each reader) were analyzed. However, the reading for MN frequency was calculated as 1000 cells [30]. The shape of MN should be round or oval with diameter size 1/3 to 1/16 from the normal nucleus and the same staining intensity and texture as the main nucleus [29].

2.6 Statistical Analysis

Data collected were analyzed using the Statistical Package for Science Version 23 (SPSS Ver. 23). Data normality of continuous variables was determined based on Shapiro Wilks. The parametric test (Independent T-test) was applied for data that normally distributed, whereas data that not normally distributed were analyzed with a non-parametric test (Mann-Whitney). Then, the data underwent further analysis for univariate, bivariate, and multivariate for each of the objectives in this study. The significant value in this study was set at $p < 0.05$.

3.0 RESULTS AND DISCUSSION

3.1 Sociodemographic Information

The demographic results of this study are shown in Table 1. The median and interquartile ranges value for age was 33 ± 4.3-year age for the exposed and 35 ± 3.3-year age for the comparative group with *p*-value 0.076. Meanwhile, the median value for height was 1.7 ± 0.06 m for exposed and 1.7 ± 0.09 m for the comparative group at $p = 0.084$. The median value for exposed and comparative group for weight was 75.0 ± 11.0 kg and 76.0 ± 13.0 kg respectively at $p = 0.761$. The median value for BMI was 26.6 ± 3.25 for the exposed group and 26.7 ± 3.17 for the comparative group. The comparison of marital status, duration of employment, and education status for the respondents were performed using a Chi-Square test. The Chi-Square test was not statistically significant for marital status ($\chi^2 = 0.051$, $p = 0.821$) and duration of employment ($\chi^2 = 2.357$, $p = 0.308$). However, education status ($\chi^2 = 45.352$, $p < 0.001$) showed statistically significant differences between the exposed group and the comparative group.

All respondents that selected in this study were male, due to the high population in traffic policemen [31]. Besides that, the selection of male respondents also to ensure the homogeneity between both study group. Furthermore, men are more associated with vehicle emission [32]. Besides, the effects of genotoxicity in women are more pronounced than in men [21]. The present study indicates that no significant difference in demographic information between traffic policemen and office workers (*p*-values > 0.05). Therefore, the expected confounder factors in this study were controlled to obtain more accurate results. The results of our study are also almost similar (age, height, weight, and BMI) to the previous study done by Muhammad *et al*., (2014) but slightly different (age and BMI) from the study done by Tan *et al*., (2017) [33, 34].

3.2 Pollutants Exposure

Table 2 presents the concentration of personal exposure of TRAP for the exposed and comparative group. The median of benzene, toluene, ethylbenzene, m,p-xylene, o-xylene, and $PM_{2.5}$ for the exposed group was 42.38, 151.37, 48.28, 78.15, 69.94, and 140 µg/m^3 respectively. Meanwhile, the median of benzene, toluene, ethylbenzene, m,p-xylene, o-xylene, and $PM_{2.5}$ for the comparative group was

7.80, 42.71, 12.08, 12.32, 22.73, and 23.00 µg/m^3 respectively. Besides, the T/B ratio and m,p – X/E were also calculated in Table 3. T/B ratio for traffic policemen and office workers was 3.4 and 5.1, respectively.

BTEX and $PM_{2.5}$ concentration levels for the present study show significant differences between exposed (traffic policemen) and comparative (office workers) groups ($p < 0.001$). Traffic police experienced four times higher exposure for BTEX and six times higher for $PM_{2.5}$ exposures compared to office workers. According to Tunsaringkarn *et al*., (2014); the T/B ratios in the range of 1.5-4.3 indicate that the BTEX source is originated from traffic emission [10]. Hence, our finding suggested that traffic policemen were exposed to air pollutants that derived from traffic emission, but not with the comparative group. Meanwhile, the value of m,p-X/E ratio for both groups indicate almost the same value. Miller *et al*., (2011) suggested that m,p-X/E ratio value more than 3.8 refer to a fresh air mass and a fresh air emission [35]. Moreover, Tiwari *et al*., (2010) suggested that lower m,p-X/E ratio refers to the high activity of photochemical reaction [36].

The predominant pollutant in this study was toluene for both exposed and comparative group. This finding was recorded similar to the previous study conducted by Kanjanasiranont *et al*., (2017) in Bangkok, Thailand [37]. The present study finding on total BTEX concentration for personal exposure among traffic policemen compare with other studies found that the concentration was lower than study finding by Kanjanasiranont *et al*. (2017), but higher than study finding by Mukherje *et al*. (2016) in Kolkata City, India [37, 38]. However, the benzene concentration in India was two times higher compared to our study, which might due to several aspects such as the structure of the city, traffic volume, and source of BTEX [39].

Table 1 Respondents demographic information

Variables	Median (IQR)		X^2	*p*
	Exposed *n* =160	Comparative *n*=149		
Age	33 ± 4.3	35 ± 3.3	-	0.07
Height (m)	1.7 ± 0.06	1.7 ± 0.09	-	0.08
Weight (kg)	75.0 ± 11.0	76. ± 13.0	-	0.76
BMI	26.6 ± 3.25	26.7 ± 3.17	-	0.96
Marital Status				
Single	28(17.5%)	27(18.5%)	0.05	0.82
Married	132(82.5%)	119(81.5%)		
Duration of employment				
< 4 years	58(36.3%)	43(28.9%)	2.35	0.30
4–10 years	55(34.4%)	62(41.6%)		
>10 years	47(29.4%)	44(29.5%)		

Table 2 Comparison of personal air pollutants exposure among the study population

Pollutants	Group	Median (µg/m^3)	IQR (µg/m^3)	*p*
Benzene	Exposed	42.38	21.76	**<0.001***
	Control	7.80	4.39	
Toluene	Exposed	151.37	78.74	**<0.001***
	Control	42.71	18.66	
Ethylbenzene	Exposed	48.28	20.02	**<0.001***
	Control	12.08	8.62	
m,p-Xylene	Exposed	78.15	37.80	**<0.001***
	Control	12.32	8.45	
o-Xylene	Exposed	69.94	34.36	**<0.001***
	Control	22.73	5.62	
$PM_{2.5}$	Exposed	140.00	41.00	**<0.001***
	Control	23.00	9.00	

*Significant at $p<0.05$

Table 3 T/B and m,p–X/E ratio between exposed and comparative group

Interspecies	Ratio	
	Exposed	Control
T/B	3.4	5.1
m,p–X/E	1.6	1.0

*T/B; toluene to benzene ratio, m,p-X/E; m,p-Xylene to ethylbenzene ratio

3.3 Micronucleus Frequency among Study Population

MN assay is a tool to monitor an early effect of chromosomal damage after exposure to carcinogenic compounds. The results of MN frequency among respondents in this study are presented in Table 4, and the images of buccal cells with MN is shown in Figure 1. Compared with the office workers, the MN frequency among traffic policemen was higher. The statistical analysis results show a significant difference in MN frequency between traffic policemen and office workers at $p < 0.001$ with z = -10.848. The median and interquartile range of MN frequency for the exposed and comparative group was 6.2±2.6 and 3.0±2.0, respectively.

The present study indicated that the MN frequency among traffic policemen was two times higher compared to office workers, due to high exposure to BTEX and $PM_{2.5}$ during working hours. The MN frequency in this study was in an agreement with a study conducted by Chaochao *et al*., (2017) in which they reported that the MN frequency of traffic policemen was higher than the comparative group [40]. Hence, the present study shows that exposure to TRAP in the Klang Valley causes chromosomal damage among urban workers especially those who actively do their activities outdoors or working outside.

In the urban environment, many compounds originated from fossil fuel combustion and vehicle emission. These compounds can act as mutagens that can cause chromosomal damage [16, 41]. Thus, people who are frequently exposed to TRAP are suspected of having high MN frequency. The previous study conducted by Recoleto and Villarino (2017) revealed that the street vendors who were exposed to a higher level of TRAP have two times higher MN frequency compared to control groups [23]. According to Wong *et al.* (2014), chemical compounds found in air pollutants can cause DNA damage that leads to the formation of MN [19]. Therefore, the present study shows that traffic policemen have higher MN frequency than those who work inside the building. In our study, the BTEX levels were recorded lower than the acceptable limit by referring to National Environment Protection (Air Toxics) Measure 2004 (Air Toxics NEPM), Australia, Environmental Protection (Air) Policy 2008 (SoQ 2008), Queensland. According to Da Rosa *et al.* (2013), exposure to the low-level carcinogenic agent can cause chromosomal damage [24]. In this study, benzene is well known as a carcinogenic agent, and $PM_{2.5}$ contain multiple compounds that are also carcinogenic to human.

Table 4 Comparison of MN frequency between traffic policemen and office workers

Variable	Median (IQR)		z	p
	Exposed	Control		
MN frequency	6.2 ±2.6	3.0±2.0	-10.848	<0.001*

*Significant at $p<0.05$, $n=215$, MN= micronucleus

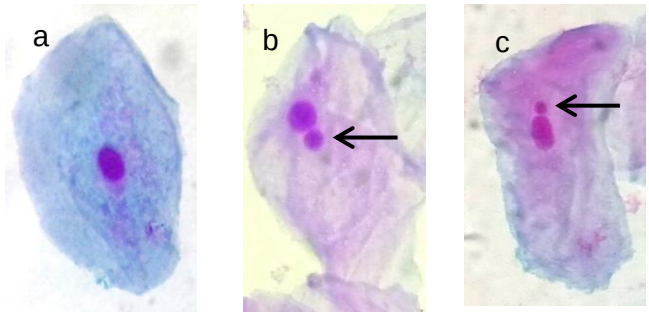


Figure 1 Images of buccal cells with micronucleus among study population viewed by a light microscope under ×1000 magnification. (a) normal cell, (b), and (c) cell with micronucleus

3.4 Respiratory Health Symptoms Characteristic

Table 5 presents the comparison of respiratory health symptoms between traffic policemen and office workers. The statistical analysis results indicate that only cough shows a significant difference among both groups ($\chi^2 = 5.465$, $p = 0.018$). Cough symptom among traffic policemen (38.1%) was higher than office workers (25.5%). Meanwhile, others symptoms; phlegm ($\chi^2 = 0.047$, $p = 0.828$), wheezing ($\chi^2 = 0.113$, $p = 0.737$) and chest tightness ($\chi^2 = 0.649$, $p = 0.421$) showed no significant difference between traffic policemen and office workers.

The results of this study suggested that exposure to high levels of TRAP can cause respiratory health symptoms among urban workers, especially those who work outdoors. Our study showed the prevalence ratio (PR) of traffic policemen that had a cough is 1.8 times higher compared to office workers. However, this finding was contradicted with the previously reported studies [33, 42]. Muhammad *et al.* (2012) found that there was no significant difference between all the variables [42]. Meanwhile, Muhammad *et al*. (2014) found that only phlegm had a significant difference between the study group and the comparative group ($\chi^2 = 8.711$, $p = 0.003$) [33]. Exposure to high-level air pollution would also increase the risk of hospitalization for chronic obstructive pulmonary disease (COPD) and respiratory mortality [43].

Table 5 Comparison of respiratory health symptoms among respondents

Variables	Exposed N=160	Control N=149	χ^2	p	PR
	Total (%)				
Cough					
Yes	61(38.1)	38(25.5)	5.46	**0.02***	1.80
No	99(61.9)	111(74.5)			
Phlegm					
Yes	36(22.5)	32(21.5)	0.05	0.83	1.06
No	124(77.5)	117(78.5)			
Wheezing					
Yes	18(11.3)	15(10.1)	0.11	0.74	1.13
No	142(88.7)	134(89.9)			
Chest tightness					
Yes	22(13.8)	16(10.7)	0.65	0.42	1.32
No	138(86.2)	133(89.3)			

$n=309$, *Significant at $p<0.05$

3.5 Association of Personal Air Pollutants Exposure with Health Effect

The association between pollutant exposures and MN frequency was analyzed using Spearman's rho correlation analysis (Table 6). The correlation analysis indicates that personal exposure for benzene ($r = 0.641$), toluene ($r = 0.570$), ethylbenzene ($r = 0.644$), m,p-xylene ($r = 0.670$), o-xylene ($r = 0.613$) and $PM_{2.5}$ ($r = 0.725$) concentration were positively correlated with MN frequency among respondents at $p < 0.001$. The present study also determined air pollutants that

influence MN frequency after controlling all confounders by performing multiple linear regression analysis (Table 7) and found that $PM_{2.5}$ and benzene were the most significant pollutants that influence MN frequency among the respondents.

In this study, $PM_{2.5}$ was one of the most important influences for MN frequency. According to Thomas *et al*. (2009), carcinogenic agents can enter the body through inhalation [29]. During inhalation, fine particles like $PM_{2.5}$ can retain in the alveolar regions in the lung and being absorbed into the bloodstream, causing inflammation, oxidative stress, and blood clots [16]. According to D'Amato *et al.* (2013), PAH is the primary compound that bounded with $PM_{2.5}$ [44]. Furthermore, PAH that found in the airborne PM comes from petroleum and diesel engine emissions [3]. Previous studies by Pereira *et al*., (2013), found that PAH exposures have a significant association with MN frequency [21]. This study found that with every increase of 1 µg/m^3 $PM_{2.5}$ exposures, the MN frequency increased by 0.018 frequencies, provided that the benzene exposure remains unchanged. When there is an increase of 1 µg/m^3 benzene exposure, there was an increase of 0.013 frequencies in MN with unchanged of $PM_{2.5}$ exposures. Also, $PM_{2.5}$ and benzene can explain 58.6% of the variance in MN frequency for personal exposure. Hence, the present study has strong evidence that $PM_{2.5}$ can cause chromosomal damage in humans and other organisms.

After considering the significant result among the respiratory health symptoms, cough symptom was chosen for further analysis. The present study also analyzed the association of pollutants and the prevalence of cough symptoms among respondents. Logistic regression was conducted for this association. The statistical analysis results are presented in Table 8. The main factors that influenced the prevalence of cough symptoms were $PM_{2.5}$ at $p = 0.013$.

Based on the findings of our study, we confirm that exposure to TRAP will lead to the development of respiratory health symptoms. The findings of the present study are in line with a previous study conducted by Willers *et al.* (2013), in which they found that exposure to PM derived from vehicle exhaust can increase the risk of respiratory health symptoms among residents living in urban areas [45]. A study by Madureira *et al*. (2015) revealed a positive association between $PM_{2.5}$ exposure with a cough and wheezing symptoms among school children in Porto, North of Portugal [46]. Jalaludin *et al.* and Kamaruddin *et al*. finding also found significant relationship between PM exposure with respiratory symptoms among school children in urban area compare to rural [47, 48]. The present study found that, in every increasing of $PM_{2.5}$ level by 1 µg/m^3, the cough symptom was three times more likely to develop and about 12.6% of cough symptom was influenced by exposure to $PM_{2.5}$ in the air.

Table 6 Correlation between personal air pollutants exposures and micronucleus frequency

Pollutants	MN (frequency)	
	r	*p*
Benzene	0.641	**<0.001***
Toluene	0.570	**<0.001***
Ethylbenzene	0.644	**<0.001***
m,p-Xylene	0.670	**<0.001***
o-Xylene	0.613	**<0.001***
$PM_{2.5}$	0.725	**<0.001***

n=195 (*traffic policemen = 105, office workers = 90*); *significant at $p<0.05$; *r* = Spearman's rho correlation coefficient

Table 7 Multiple linear regression for the association between personal air pollutants exposure with micronucleus frequency after controlling the confounders

Pollutants	*B*	*SE*	*β*	*t*	*p*
Constant	2.551	0.151	-	16.903	0.000
$PM_{2.5}$	0.018	0.002	0.649	8.400	**<0.001***
Benzene	0.013	0.006	0.161	2.084	**0.039***

n=195 (*traffic policemen = 105, office workers = 90*); *Significant at $p<0.05$; Adjusted $R^2 = 0.586$; B = Regression Coefficient; SE = Standard Error; *β* = standardized beta; *t* = *t*-test statistic. Adjusted for age, BMI, duration of employment and smoking hobbit.

Table 8 Logistic Regression for Personal Air Pollutants Exposure with Cough after Controlling the Confounders

Pollutants	*B(SE)*	*p*	*PR*	*95% CI*
Constant	-1.30(0.23)	0.000	0.27	-
Benzene	-0.65(1.02)	0.522	0.52	0.07 – 3.84
Toluene	-0.82(1.00)	0.413	0.44	0.06 – 3.14
Ethylbenzene	1.70(1.00)	0.090	5.49	0.77 – 39.46
m,p-Xylene	0.57(1.19)	0.632	1.77	0.17 – 18.12
o-Xylene	-1.13(0.77)	0.138	0.32	0.07 – 1.44
$PM_{2.5}$	1.20(0.48)	**0.013***	3.30	1.29 – 8.44

n=195 (*traffic policemen = 105, office workers = 90*); CI = 95% Confidence Interval; *B* = Regression Coefficient; *S.E* = Standard Error; Nagelkerke R Square = 0.126; *Significant at $p<0.05$. Adjusted for age, BMI, duration of employment and smoking hobbit.

4.0 CONCLUSION

The present study strongly suggests that exposure to TRAP (BTEX and $PM_{2.5}$) can cause chromosomal damage as measured by MN frequency and increase the prevalence of respiratory health symptoms among traffic policemen in Klang Valley. Urban workers who work outside the building has a higher percentage of exposure to air pollution compared to workers working in the building. The result for the personal exposure concentration for total BTEX and $PM_{2.5}$ was 390.12 µg/m^3 and 140 µg/m^3 respectively among traffic policemen. Meanwhile, the results for personal air pollutant exposure among office workers were 97.64 µg/m^3 and 23.00 µg/m^3, respectively. The results of this study show that traffic policemen are exposed to TRAP three times higher compared to office workers. Furthermore, the present study also found that the MN frequency between traffic policemen (6.2±2.6) and office workers (3.0±2.0) has a significant difference. The analysis of respiratory health symptoms, as determined by a questionnaire, recorded a significant difference in terms of cough symptoms among policemen and office workers ($\chi^2 = 5.465$, $p = 0.018$). In addition, there was a positive correlation between air pollution exposure and MN frequency (benzene; r = 0.641, toluene; r = 0.570, ethylbenzene; r = 0.644, m,p-xylene; r = 0.670, o-xylene; r = 0.613 and $PM_{2.5}$; r = 0.725) at $p<0.01$. Furthermore, $PM_{2.5}$ and benzene were the most important pollutants that induced the formation of MN among respondents in this study. This finding indicated that outdoor workers who have been exposed to a higher level of TRAP had an increased rate of MN formation due to chromosomal damage compared to the office workers. Hence, traffic policemen are at risk of cancer due to higher MN frequency. In short, MN frequency in buccal mucosa cells can be used as a useful biomarker for genotoxicity of TRAP exposure among urban workers. $PM_{2.5}$ was the main pollutant that influenced cough symptoms among respondents. However, other pollutants from TRAP must also be further investigated in a larger sample size for better understanding regarding the effect of air pollutant's exposure and health outcome among urban residents.

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