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Genotoxic Effects of Exposure to Urban Traffic Related Air Pollutants on Children in Klang Valley, Malaysia

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ABSTRACT

Traffic-related air pollutants (TRAPs) are currently increasing due to the increment of vehicle numbers in Malaysia. The emission of pollutants from these vehicles have genotoxic properties that can potentially induce genetic damage in human. In this study, micronuclei assay is used to determine the potential genotoxic exposure by assessing the presence of micronuclei frequency (MN) in buccal cells. The specific objective of this study is to determine the association between TRAPs and frequency of MN among school children in Klang Valley. A comparative cross-sectional study was conducted among primary school children (9–11 years old) in high-density traffic area (n = 94, Kuala Lumpur) and low-density traffic area (n = 94, Hulu Langat). A questionnaire was distributed to the parents to obtain respiratory symptoms information. Buccal cells were analyzed to determine the frequency of micronuclei. Air quality assessment was carried out in a total of 6 schools (consisted of exposed and comparative groups) by using TSI DustTrak DRX Aerosol Monitor 8534 for measurement of $PM_{2.5}$ and PM_{10}, LaMotte's Model BD Air Sampling Pump for measurement of Nitrogen dioxide (NO_2) and Sulphur dioxide (SO_2), ppbRAE 3000 for total Volatile Organic Compound (TVOC) and TSI Q-TRAK 7565 for measurement of Carbon dioxide (CO_2) and Carbon monoxide (CO). The concentrations of PM_{10}, $PM_{2.5}$, NO_2, SO_2, TVOC and CO at exposed schools were significantly higher ($p < 0.005$). compared to those in comparative schools. All pollutants were significantly associated ($p < 0.001$) with respiratory symptom phlegm and MN frequency. Additionally, the MN frequency in the exposed group was significantly higher ($p < 0.001$) than in the comparative group. All in all, this study demonstrated that air pollutants, especially NO_2 and CO, have significantly influenced the MN frequency among children in primary schools. This study suggested that exposure to TRAPs among Malaysian school children has increased the risk for respiratory complications with the formation of MN.

Keywords: TRAPs; Urban traffic pollutants, Genotoxicity; Micronuclei frequency; Children.

INTRODUCTION

Traffic-related air pollutants (TRAPs) exposure is a public health concern as it causes many diseases each year in Malaysia (Ismail *et al.*, 2019). The increasing number of vehicles remains the most significant source of air pollution in many urban areas (Shuhaili *et al.*, 2013; Wong *et al.*, 2017), especially in Klang Valley (Rahman *et al.*, 2017). Klang Valley has been identified as the primary source area of pollutant emission since it started to become the central development area in Malaysia (Latif *et al.*, 2014).

Urban air pollution can be considered as the potential contributor towards the overall air pollution in Malaysia in addition to transboundary haze pollution (Rahman *et al.*, 2017; Kuwata *et al.*, 2018). Due to the increased number of motor vehicles and manufacturing industries in urban areas, the release of toxic gases such as nitrogen dioxide (NO_2), sulphur dioxide (SO_2), carbon monoxide (CO) and carbon dioxide (CO_2) have also increased. This unfavourable condition has caused severe health impacts especially on children with respiratory symptoms such as wheezing, coughing, chest tightness and phlegm (Arifuddin *et al.*, 2019; Ismail *et al.*, 2019; Suhaimi *et al.*, 2020). The increase in traffic emission is one of the major sources of pollutants in high-density urban cities, and stagnated airflow due to densely packed tall buildings makes the dispersion of air pollutants difficult (Tajudin *et al.*, 2019). All in all, the combination of these conditions lead to frequent reports of higher level of air pollutants in urban areas.

In Malaysia, Khan *et al.* (2015) performed fixed-site monitoring on the levels of particulate matter (PM_{10}) and

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trace gases at an urban city of Kuala Lumpur. They have observed a positive correlation between PM_{10} level and the number of motor vehicles, as well as activities of biomass burning, particularly from Sumatra, Indonesia, happened during the southwest monsoon. The main sources of the trace gas emissions were from motor vehicles, manufacturing industries, and densely populated cities (Dominick *et al.*, 2012). In urban areas such as the Klang Valley region, the concentrations of CO, NO_2, and SO_2 gases were mainly influenced by the heavy road traffic (Azmi *et al.*, 2010; Abdullah *et al.*, 2012). On the other hand, the concentration of secondary gas such as ozone (O_3) was mainly related to local and regional factors such as the presence of NO_x and Volatile Organic Compounds (VOCs) as O_3 precursors, and their reactions with ultraviolet radiation from sunlight (Azmi *et al.*, 2010).

Rapid urbanisation that resulted in the increment of traffic density has become a public concern. For example, children residing near condensed urban traffic area are having constant exposure to urban air pollution. As children have physiological features such as higher inhalation rates and immature respiratory system when compared to adults, they are more vulnerable to traffic-based gas pollution which will induce harmful health effects (Beatty *et al.*, 2014). Exposure to environmental air pollution started from childhood could increase the risk of DNA damage progression and eventually has a higher chance to develop cancer during their adulthood (Carpenter and Bushkin-Bedient, 2013; Daud *et al.*, 2018).

The mechanism of oxidative stress refers to the human biological system that interacts with airborne particulate matter by direct production of reactive oxygen species (Risom *et al.*, 2005). Oxidative stress is the disproportion between antioxidant capacity and the production of free radical that causes DNA damage (Scandalios, 2005). Micronuclei (MN) frequency that measures chromosome loss and chromosomal breakage have been extensively used to assess chromosomal instability and potential genotoxic exposures (Fenech *et al.*, 2002). Specifically, by using micronuclei assay in exfoliated buccal cells, monitoring for environmental and occupational genotoxins exposure in human has become an easy method without the requirement to perform cell tissue culture (Burgaz *et al.*, 2002; Fenech *et al.*, 2002).

Chen *et al.* (2015) stated that adverse respiratory health impact affecting children living in urban area may be influenced by the exposure to air pollutants produced by vehicles emission. In Malaysia, a study by Arifudin *et al.* (2019) portrayed the relationship of high-density traffic with the air pollutants emitted and the results indicated that the pollutants concentration in air were depending on the distance from the road and number of vehicles on the road. The study has shown that the children attending school near heavy traffic areas in Kajang have higher prevalence of respiratory symptoms compared to primary school children attending school near low-density traffic area in Hulu Langat.

To focus on traffic as the main contributor to urban air pollution, the association between urban traffic exposure and genotoxicity biomarker has to be investigated by conducting MN assay among children, especially in South East Asia region. Therefore, this study aims to determine the associations between TRAPs (i.e., $PM_{2.5}$, PM_{10}, CO, NO_2, SO_2, TVOC) exposure and DNA damage as well as respiratory health symptoms among children attending schools located at high and low traffic densities in Klang Valley area.

METHODS

Study Area

This was a cross-sectional comparative study conducted among primary school children in Kuala Lumpur as exposed group (n = 94) and Hulu Langat, Selangor as comparative group (n = 94). Exposed group was primary school children attending schools located at high-density traffic area while comparative group was primary school children located at low-density traffic area (Fig. 1). High-density traffic area can be categorized as area with an average daily traffic volume of more than or equal to 18000 vehicles located 500 m from highways (Brauer *et al.*, 2013). Low-density traffic area was considered as area with minor roads, characterized by low traffic volume with an average of 2800 vehicles/day (Elhadi *et al.*, 2017).

Ethics Approval

This study was approved by Ministry of Education Malaysia and reviewed by Ethics Committee for Research Involving Human Subjects Universiti Putra Malaysia (UPM) with the reference number of JKEUPM-2018-324 and JKEUPM-2018-376 prior to data collection.

Selection of Respondents

A total of 188 Malay primary school children in high traffic area (Kuala Lumpur) and low traffic area (Hulu Langat) aged 9 to 11 years old were selected in this study. The selection of respondents was conducted through stratified random sampling method. The respondents were stratified according to their ages. The name list of primary school children was obtained from school administration from each school. The students who fulfilled the inclusion criteria and have received permission from parents or guardian were randomly chosen as respondents for this study. The inclusion criteria were children aged between 9 to 11 years old and Malay children in order to homogenize the respondents. Children with family history of cancer, chronic disease or disorder such as asthma, bronchitis as well as children that had undergone radiotherapy or chemotherapy within a year or X-ray within 3 months were excluded in this study. Parental permission was mandatory for participation in this study.

Measurement of TRAPs

DustTrakTM DRX Aerosol Monitor 8534 were used for measurement of $PM_{2.5}$ and PM_{10}, Q-TrakTM Indoor Air Quality Monitor 7575 for measurement of CO, CO_2, relative humidity and temperature, TSI VelocicalC Plus Model 8386 for measurement of air velocity and LaMotte's Model BD Air Sampling Pump for measurement of NO_2 and SO_2. Measurement were taken at 1 meter above the floor which represents children breathing area and 1.5 meter away from windows and door. Air monitoring was taken on daily basis for 6 hours of school hours from 7.20 am until 1.20 pm.

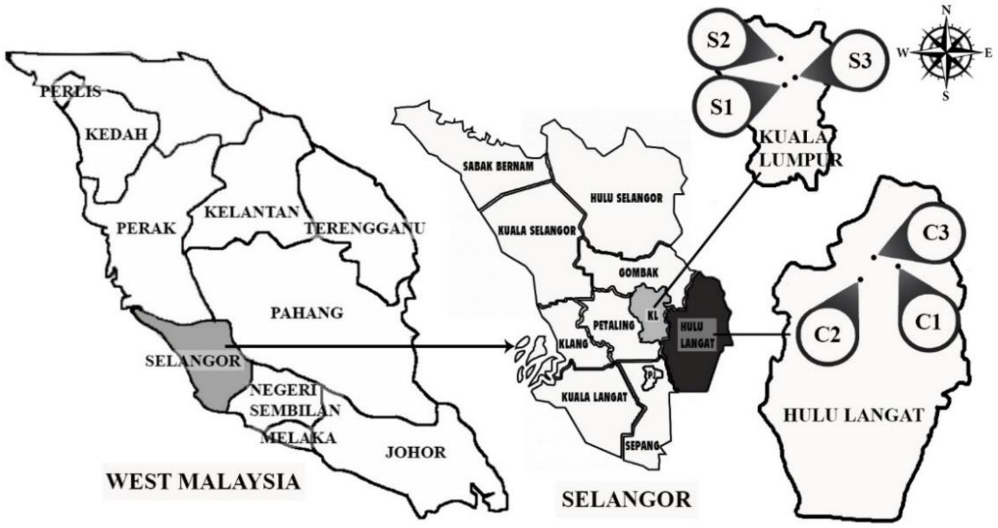

Fig. 1. Location of study groups.

Traffic Count Survey

Traffic count surveys were conducted at two-time intervals per day which were from 7.00 am to 7.30 am and from 1.00 pm to 1.30 pm when school children arrived at and left school. This method was adapted from previous study conducted by Lee *et al.* (2015). The type of vehicle was classified according to the group of light duty vehicle (motorcycle), medium duty vehicle (cars and taxis) and large duty vehicles (buses and good vehicles).

Questionnaire

A modified questionnaire based on two standardized international questionnaire ATS-DLD-78-C by ATS was used for data on sociodemographic background and school and home environment. While questionnaire on asthma, allergies and respiratory symptoms were obtained from International Study of Asthma and Allergies in Childhood (ISAAC). The questionnaires then were distributed to parents to be filled in.

Micronuclei Frequency

Children were requested to rinse mouth thoroughly with mineral water to remove exfoliated dead cell prior to sampling. A sterile cytology brush was used to collect the cells by gently scrapping against both inner surface of the cheeks wall. For micronuclei frequency analysis, the cells were suspended in warm PBS (phosphate buffered saline solution) with pH of 7.5 and then were stored in freezer (–20°C). The cells were smeared onto glass slides and subsequently fixed in cold 0.1 M phosphate buffer (pH 7.5) with 1% glutaraldehyde for 20 minutes. Following staining the slides with Schiff reagent for an hour, the slides were counterstained with 0.1% Fast Green for 20 seconds. Finally, the slides were observed under light microscope with 40x magnification (Fig. 2).

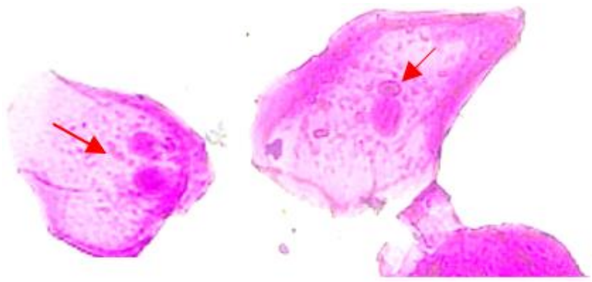

Fig. 2. Arrow indicates presence of micronuclei in buccal exfoliated cell under 40x magnification.

Statistical Analysis

The Statistical Package for Social Science (SPSS) Version 22.0 was used to analyze the data. A normality test was performed initially to evaluate the data distribution among the exposed and comparative group. The univariate statistical testing was used for descriptive analyses of sociodemographic, air pollutants exposure and MN frequency. Bivariate statistical testing using Mann-Whitney test were performed to compare the median of air pollutants concentration and micronuclei frequency as the data were not normally distributed. Chi-Square analysis were executed to assess the respiratory symptoms. Meanwhile, multiple linear regression was performed to evaluate the associations between air pollutants concentrations and MN frequency.

RESULTS AND DISCUSSION

Socio-demographic Data

A total of 230 consent forms and questionnaires were distributed to children's parents or guardians in exposed and comparative group. The response rate for returned questionnaires and approved consent form by parents or guardians was approximately 82% (188), with 94 children from high-density traffic area, while another 94 children from low-density traffic area. For both exposed and comparative group, female respondents precedes male respondents in numbers with 58.5% (55) to 41.5% (39) of male respondents in exposed group whereas 57.4% (54) to 42.6% (40) of male respondents in comparative group respectively. As shown in Table 1, most of the respondents in exposed group (80.9%)

Table 1. Respondents' outdoor residential background and exposure to indoor pollutant sources among study groups.

Variables	Exposed Group (n = 94)	Comparative Group (n = 94)	χ^2 value	p-value
	Number (%)	Number (%)		
Housing area				
Urban	76 (80.9)	2 (2.1)	141.440	**< 0.001***
Sub-urban	16 (17.0)	15 (16.0)		
Rural	2 (2.1)	77 (81.49)		
Distance of house from main road				
< 100 meters	18 (19.1)	12 (12.8)		
100–299	21 (22.3)	4 (4.3)	21.546	**< 0.001***
300–499	11 (11.7)	6 (6.4)		
> 500 meters	44 (46.8)	72 (76.6)		
ETS exposure				
Yes	70 (74.5)	55 (58.5)	5.371	0.020
No	24 (25.5)	39 (41.5)		
Fuel for cooking				
Gas	78 (83.0)	69 (73.4)	4.051	0.132
Electric	1 (1.1)	0 (0)		
Gas and electric	15 (16.0)	25 (26.6)		
Mosquito repellent usage				
Yes	58 (61.7)	64 (68.1)	0.841	0.359
No	36 (38.3)	30 (31.9)		

Chi-square test.
* Significant at p < 0.05.
N = 188.

were living in urban area whereas most of the respondents in comparative group (81.49%) were living in rural area. It was also recorded that half of respondents in exposed group (53.1%) resided less than 500 meters from the main road, while 76.6% of the respondents from comparative group living exceeding 500 meters from the main road. Both housing area and the distance of respondents' house from main road in exposed and comparative group showed significant difference ($p < 0.001$). Meanwhile, more than 50% respondents in both groups were exposed to environmental tobacco smoke (ETS) with the percentage of 74.5% and 58.5% in exposed and comparative group, respectively. Majority of respondents' families used gas for cooking purposes, in which 83% of them from exposed group and 73.4% from comparative group. Moreover, this study found that 61.7% of the respondents' families in exposed group used mosquito repellents, while 68.1% of respondents' families in comparative group used mosquito repellents.

TRAPs in High and Low Traffic Density Areas and Vehicle Count

The average of 6 hours TRAPs concentrations in exposed and comparative schools for five school days were shown in Fig. 3, while the comparison of TRAPs concentrations between two groups was tabulated in Table 2. The result demonstrated that TRAPs concentrations in high- density traffic area were significantly higher compared to low-density traffic area. These findings were influenced by the location of the primary schools of exposed group that are located in high- density traffic area which has an average daily traffic volume of more than or equal to 18000 vehicles or 100 m of major roads with an average daily traffic volume of more than or equal to 15000 vehicles. On the contrary, the comparative group for this study focuses on primary schools located at low-density traffic with an average of 2800 vehicles/day and located away from traffic congestion. The result was corroborated by result of previous local study which stated that there was a significant difference ($p < 0.001$) between the concentrations of TRAPs in school located at high-density traffic area compared to less busy area (Arifuddin *et al.*, 2019).

To further explore relationship between TRAPs concentrations and traffic count at schools, correlation analysis was conducted, and the results are showed in Table 3. We observed a significant strong correlations of traffic count with PM_{10}, $PM_{2.5}$, NO_2, CO and TVOC ($r > 0.8$, $p < 0.05$). Based on the traffic count survey, the number of medium size vehicles was the highest among light and heavy-duty vehicles. A study by Zuurbier *et al.* (2010) stated that medium size vehicles produced low concentrations of traffic-related air pollutants compared to large vehicles, however the high number of medium size vehicles was responsible for the high air pollutants emission. In addition, throughout both peak hours (morning and afternoon) of schooling hours, there was a high number of medium size vehicles idling nearby primary school compound in high-density traffic area due to parents waiting for their children.

Comparison of MN Frequency between School Children in High- and Low-density Traffic Areas

The results presented in Table 4 showed that children in high-density traffic area had a higher median of MN frequency [18.5(5.5)] compared to children in low-density traffic area [0.50 (1.0)]. The Mann-Whitney U test showed a significant difference in the value of MN Frequency (z =

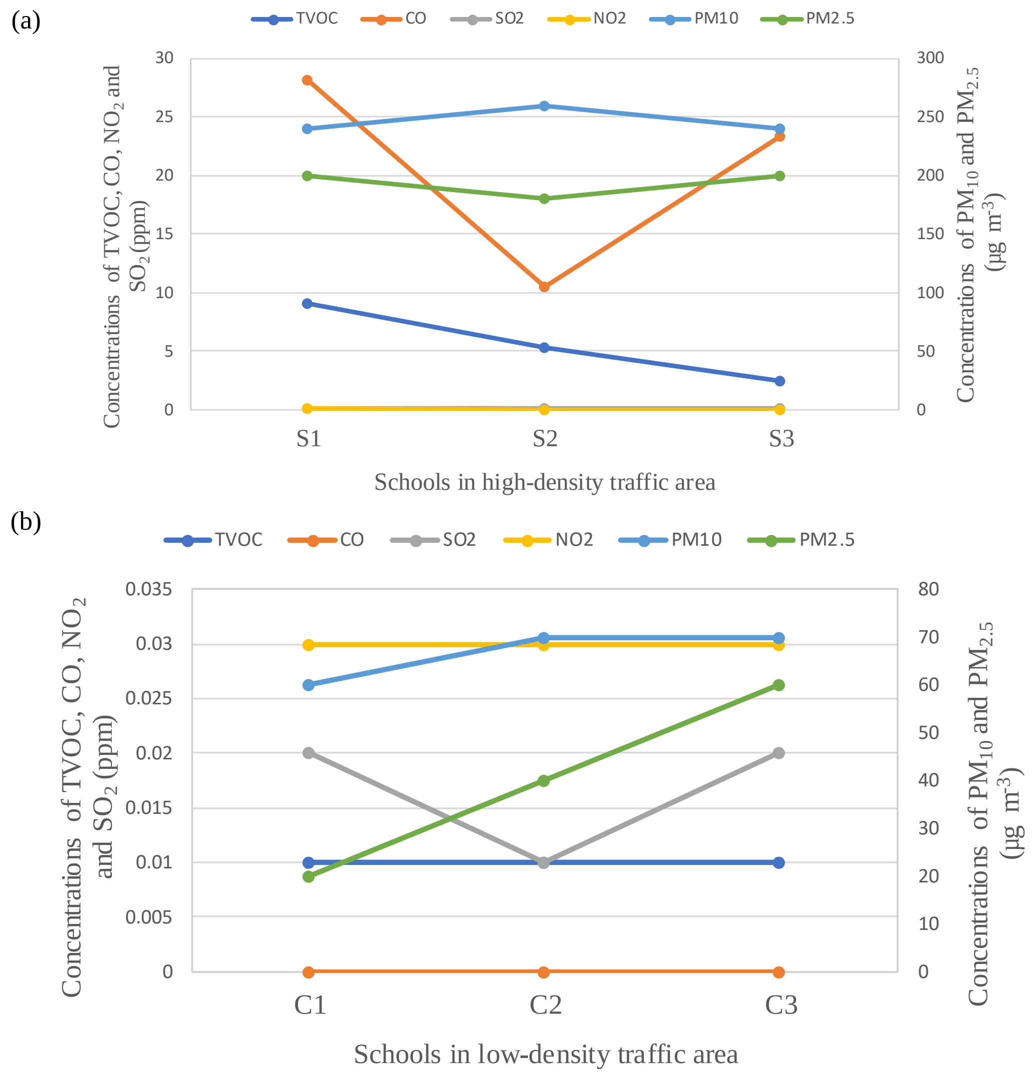

Fig. 3. Average of TRAPs concentrations (6 hours) in exposed and comparative schools for five school days.

Table 2. Comparison of TRAPs between exposed and comparative groups.

Variables	Exposed group (n = 9)	Comparative group (n = 9)	t-value/z-value	p-value
	Mean (SD)/Median (IQR)			
PM_{10} ($\mu g\ m^{-3}$)[b]	246.67 (11.55)	66.67 (5.77)	–2.023	0.043*
$PM_{2.5}$ ($\mu g\ m^{-3}$)[b]	193.33 (11.55)	40.00 (20.00)	–11.500	< 0.001*
NO_2 (ppm)[a]	0.24 (0.10)	0.03 (0)	–3.623	0.022*
SO_2 (ppm)[a]	0.09 (0.01)	0.02(0.01)	–6.709	0.003*
CO (ppm)[b]	23.32 (9.11)	0 (0)	–3.931	0.017*
TVOC (ppm)[b]	5.14 (10.91)	0.01 (0)	–2.087	0.037*

[a] T-test, [b] Mann-Whitney U Test.
* Significant at $p < 0.05$.

–11.946, p < 0.001), among children in high- and low-density traffic areas. Similar finding was observed in 269 children aged 6 to 17 years old in Calcutta where the urban children exhibited higher MN frequency than children in rural area (0.22 vs. 0.17%, $p < 0.05$) (Lahiri *et al.*, 2000). Another study on genotoxicity of air pollutants in children under 7 years of age reported a significant difference between MN frequency in children exposed to biomass burning than those in control area (0.12% vs. 0.02%) (Sisenando *et al.*, 2012). Huen *et al.* (2006) evaluated chromosomal damage in children and adult using MN assay, reported that children are more susceptible to traffic-related air pollution. Another study conducted on Turkish children from two schools at urban-traffic and suburban sites showed a significantly higher MN frequency in children exposed to urban traffic exposure during summer (2.73 ± 1.98%) compared to exposure

Table 3. Correlation between TRAPs with traffic count in schools.

Pollutants	*r*-value	*p*-value
PM_{10} (µg m^{-3})	0.883	0.020
$PM_{2.5}$ (µg m^{-3})	0.841	0.036
NO_2 (ppm)	0.926	0.008
SO_2 (ppm)	0.794	0.059
CO (ppm)	0.880	0.021
TVOC (ppm)	0.941	0.005

Spearman's Rho.
r-value indicates strong correlation at $r > 0.8$.

Table 4. Comparison of the MN frequency among school children in high- and low-density traffic area.

Variables	High-density traffic area ($n = 94$)	Low-density traffic area ($n = 94$)	z-value	*p*-value
	Median (IQR)			
MN frequency	18.5(5.5)	0.50 (1.0)	–11.946	< 0.001*

Man-Whitney U Test.
* Significant at $p < 0.05$.

during winter season (1.87 ± 1.66%). In contrast, children in comparative group had low MN frequency (Demircigil *et al.*, 2014). Comparison between polluted and less polluted area showed that children exposed to pollution have a higher frequency of micronuclei. It can be deduced that long term exposure to high concentrations of air pollutants could contribute to cumulative chromosomal damages which might elevate the cancer risk among children living in proximity to polluted area (Sopian *et al.*, 2020).

Prevalence of Respiratory Symptoms among School Children in High and Low Traffic Density Areas

As shown in Fig. 4, there were four parameters of respiratory health symptoms that were assessed which were wheezing, cough, chest tightness and phlegm. 14.9% of children from exposed group and 13.8% of children from comparative group experienced cough. Next, 9.2% of children from exposed group and 3.2% of children from comparative group reported experienced wheezing. Symptom such as phlegm was the most common among studied groups which reported 34% of children for exposed group but only 1.1% of children from comparative group. Chest tightness recorded 5.3% of children from exposed group and 3.2% of children from comparative group.

The result demonstrated that the prevalence of respiratory symptoms was generally higher among children in high-density traffic area compared to children in low-density traffic area. Based on the statistical analysis, a significant difference was found only on the reported respiratory symptom phlegm ($p < 0.001$, PR = 0.021, 95% CI = 0.003–0.156).

Statistically, there were significant associations between phlegm with PM_{10}, $PM_{2.5}$, NO_2, SO_2, CO and VOC exposure at primary schools ($p < 0.001$, PR = 0.021, 95% CI = 0.003–0.156). The finding was supported by study by Migliore *et al.* (2009), where majority of TRAP have strong associations with phlegm or cough (high traffic density OR = 1.24; 95% CI: 1.04, 1.49). Similar finding was observed in study by Daud *et al.* (2018) where phlegm recorded the highest prevalence occurred among the exposed group (PR = 9.66; 95% CI 2.10–44.46).

Increased exposure to air pollutants from road traffic, industrial, and indoor sources may contribute to phlegm symptoms among children in exposed group (Daud *et al.*, 2018). The finding in this study such that location of school near high-density of traffic has significantly contributed to high prevalence of respiratory health symptoms also supported by the findings from other local studies (Noor Hisyam and Juliana, 2014; Anis *et al.*, 2014; Choo *et al.*, 2015; Wesley and Jalaludin, 2015) where prevalence of respiratory health symptoms was higher among children in the urban area as compared to the suburban or rural area.

Correlation between TRAPs with MN Frequency and Factor Influenced the MN Frequency among Children

Based on Table 5, a statistically significant correlations of MN with concentration of air pollutants were found for PM_{10} (r =0 .806, $p = 0.001$); $PM_{2.5}$ ($r = 0.752$, $p = 0.001$); TVOC ($r = 0.820$, $p = 0.001$); NO_2 ($r = 0.820$, $p = 0.001$); SO_2 ($r = 0.849$, $p = 0.001$) and CO ($r = 0.834$, $p = 0.001$). The correlation results (*r*-value) of PM_{10}, $PM_{2.5}$, TVOC, NO_2, and CO were greater than 0.7 suggested a strong positive correlation between these TRAPs and genotoxicity of buccal cells. Meanwhile, no apparent correlation was found between MN frequency and other exposure variables (ETS exposure, distance of house from major road, mosquito repellent usage, fuel for cooking) investigated via the questionnaire.

Multiple linear regression was performed to assess the contributing factors of micronuclei frequency among the respondents. Independent variables that have the *p* value < 0.001 in correlation test were included in the multiple linear regression analysis. As shown in Table 6, unstandardized (B) coefficients indicated the predicted change in MN frequency with a 1-unit increased in PM_{10}, $PM_{2.5}$, NO_2, SO_2 and CO concentrations and standardized coefficients (β) indicated the predicted change in standard deviations in MN frequency with a 1-unit increased in TRAPs. Based on regression analysis, we found that NO_2 and CO were the most significant air pollutant predictors to induce the formation

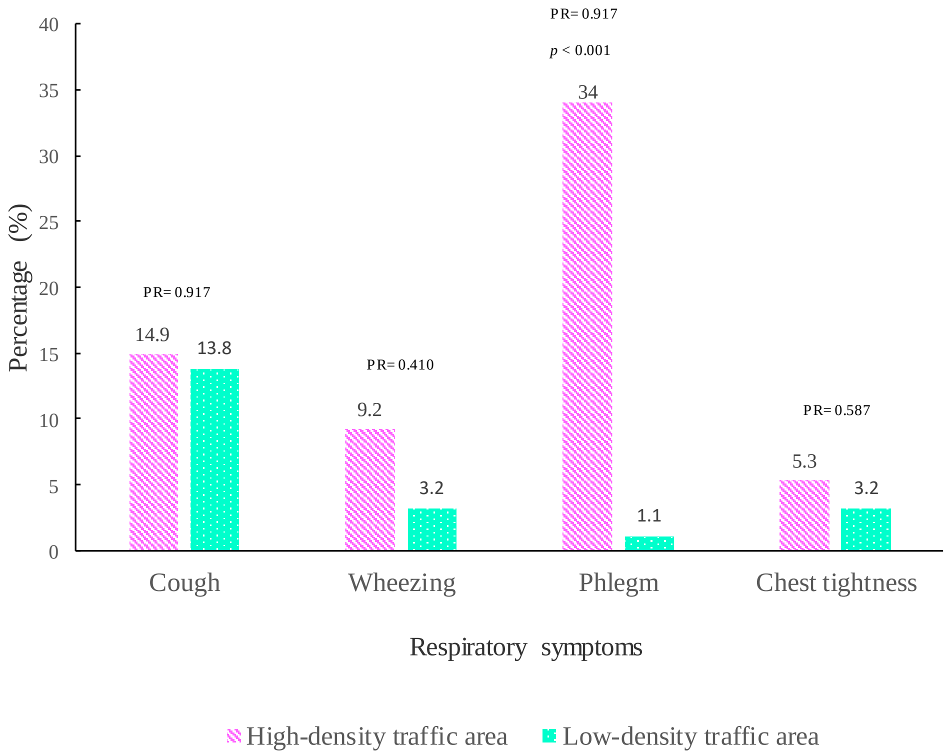

Fig. 4. Prevalence of respiratory symptoms among school children in high- and low-density traffic area.

Table 5. Correlation between TRAPs, ETS exposure, distance of house from main road, mosquito repellent usage and fuel for cooking with MN frequency in exposed and comparative groups.

Variables	MN frequency (n = 188)	
	r-value	*p*-value
PM_{10}	0.806	< 0.001*
$PM_{2.5}$	0.752	< 0.001*
NO_2	0.820	< 0.001*
SO_2	0.849	< 0.001*
CO	0.834	< 0.001*
TVOC	0.820	< 0.001*
ETS exposure	–0.169	0.020
Distance of house from main road	–0.017	0.818
Mosquito repellent usage	0.067	0.362
Fuel for cooking	–0.119	0.103

Spearman's Rho.
* Significant at $p < 0.01$.
r-value indicates strong correlation at $r > 0.7$.

Table 6. Factors that influenced MN frequency among children.

Variables	B	β	S.E	*p*-value	95% CI
Constant	–2.201	-	24.795	0.089	–51.122–46.720
$PM_{2.5}$ (µg m^{-3})	0.175	0.490	0.010	0.171	0.155–0.196
PM_{10} (µg m^{-3})	5814	0.009	109.122	0.958	209.485 – 221.113
NO_2 (ppm)	5.006	0.463	2.292	**0.030***	0.484–9.527
SO_2 (ppm)	16.041	0.040	57.413	0.780	97.235–129.317
CO (ppm)	66.481	0.540	10.101	**< 0.001****	46.551–86.410
TVOC (ppm)	25.154	0.549	1.324	0.341	22.506–27.766

* significant at $p < 0.05$, **p < 0.001, 95% CI = 95% Confidence Interval,
B = Unstandardized Coefficient, β = Regression Coefficient, S.E = Standard Error, R^2 = 0.914 (Adjusted for housing area, distance of house from major roads, ETS exposure, mosquito repellent usage and fuel for cooking).

of micronuclei among studied respondents with R^2 value of 0.914, after controlling all possible confounders such as distance of the homes from main road, ETS exposure, mosquito repellent usage as well as fuel for cooking.

The result corroborated the previous studies by Ceretti *et al.* (2014) where a significant association was found between MN frequency and fine particulate exposure in urban area, similar to findings by Sopian *et al.* (2020) conducted on children in industrial area. In urban area, gaseous and particulate pollutants interact with each other, thereby enhancing the genotoxic effects on the living organisms. Upon high reactivity of NO_2, its exposure can lead to reaction between the particulate with lipids, proteins, and nucleic acids (Han *et al.*, 2013). In a study of genotoxicity evaluation using *in vivo* MN assay, Han *et al.* (2013) demonstrated the increasing inhalation exposure of rats to NO_2 had significantly induced higher formation of MN frequencies. This can be explained by the metabolite reaction of nitrate and nitrite as it can lead to DNA impairment and resulting in development of mutation or cancer. Koehler *et al.* (2011) suggested that NO_2 induced the formation of micronuclei frequency or permanent DNA impairment on human nasal epithelial cell at 0.1 ppm at an exposure time of 3 h.

Outdoor CO mainly comes from vehicle emissions and heavy traffic congestion areas typically shows high concentrations of CO. CO binds to haemoglobin, forming carboxyhaemoglobin (COHb) and prolonged exposure to CO may cause CO poisoning especially among children who have underdeveloped lung. This statement is in agreement with Estrella *et al.* (2005) which proved that school in high congestion of traffic tend to have higher reported cases of CO toxicity in comparison with school in lower traffic congestion. Ślęzak *et al.* (2014) showed that there was a significant relationship between intoxicated (COHb) patients with formation of micronuclei. Hence, the finding in this study showed potential genotoxicity of carbon monoxide especially from traffic emission at primary schools located at high traffic density.

A fact that could be considered a limitation of the present study was other toxic pollutants emitted from motor vehicles were not investigated in this study. It is difficult to ascribe a single pollutant such as NO_2 and CO as the causality of the observed findings. NO_2 and CO may represent a proxy variable of fresh automotive emissions most probably associated with other non-measured carcinogenic air pollutants such as polycyclic aromatic hydrocarbon (PAHs) and benzene that are strongly associated with genotoxic effects. PAHs and benzene can possibly induce the MN formation through additive and synergistic effects (Sopian *et al.*, 2020). Following PAHs exposure through inhalation, it could inhibit the methylation of DNA and induce the formation of benzo(a)pyrene diol epoxide (BPDE)-DNA adducts. The failure of the cell to perform DNA adduct and DNA strand break repair can contribute to formation of micronuclei (Fenech *et al.*, 2016).

The measurement of biomarker only is considered inadequate in associating air pollutants with MN frequency in children. The influence of demographic factors such as lifestyle and dietary pattern of children (supplementation with B vitamins or antioxidant, meat consumption, fruit consumption and alcohol intake) as well as information on infectious illnesses are also significant to interpret MN assay results (Holland *et al.*, 2011). Data on cotinine concentration should not be ignored as variables to control confounding of ETS exposure.

CONCLUSIONS

In conclusion, the present study suggested that concentration of TRAPs especially PM_{10}, $PM_{2.5}$, NO_2, SO_2, TVOC and CO were significantly higher ($p < 0.005$) at schools in high-density traffic area compared to schools in low-density traffic area. Overall, this study concluded that TRAPs exposure was significantly associated ($p < 0.001$) with respiratory symptom phlegm and MN frequency. The findings, after controlling for confounding factors (housing area, distance of house from main road, ETS exposure, mosquito repellent usage, fuel for cooking), strongly demonstrated that exposure to NO_2 and CO probably increases the risk of chromosomal damage for children living in proximity to heavy traffic area. However, future research should be conducted on quantifying other carcinogenic pollutants such as PAHs and toxic gases (benzene, toluene, ethylbenzene, xylenes) to evaluate comprehensively the combine effects of exposure to urban traffic air pollutants on genotoxic effects in children. Other limitations must be noted, including the dependency on self-reported information for both symptoms and exposure which may cause the validity of information acquired in the survey to be over-reported or inaccurate. In addition to exposure assessment at school, future research should focus on the collection of data based on home exposure assessment, as children spend most of their time at home. All in all, the present study and future recommendations are important in estimating the synergistic effect of both exposures towards children respiratory health and the genotoxic effect that could lead to chronic disease such as cancer.

There is a need to implement strategies that may reduce children's exposure to pollutants from vehicle emission. Hence, the implementation of anti-idling and idle reduction policies at school is highly recommended. In addition, it is advisable for school management to relocate the drop-by and waiting sites for parents to an area that is away from the school compound. Vegetation may also help in reducing the concentrations of TRAPs by using of multiple vegetation type such as a combination of bushes and trees.

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REFERENCES

Abdullah, A.M., Samah, M.A.A. and Jun, T.Y. (2012). An overview of the air pollution trend in Klang Valley,

Malaysia. *Open Environ. Sci.* 6: 13–19. https://doi.org/10.2174/1876325101206010013
Anis, S.K., Jalaludin, J. and Choo, C.P. (2015). Indoor air quality and its association with respiratory health among Malay preschool children in Shah Alam and Hulu Langat, Selangor. *Adv. Environ. Biol.* 9: 17–26.
Arifuddin, A.A., Jalaludin, J. and Hisamuddin, N.H. (2019). Air pollutants exposure with respiratory symptoms and lung function among primary school children nearby heavy traffic area in Kajang. *Asian J. Atmos. Environ.* 13: 21–29. https://doi.org/10.5572/ajae.2019.13.1.021
Azmi, S.Z., Latif, M.T., Ismail, A.S., Juneng, L. and Jemain, A.A. (2010). Trend and status of air quality at three different monitoring stations in the Klang Valley, Malaysia. *Air Qual. Atmos. Health* 3: 53–64. https://doi.org/10.1007/s11869-009-0051-1
Beatty, T.K.M. and Shimshack, J.P. (2014). Air Pollution and children's respiratory health: A cohort analysis. *J. Environ. Econ. Manage.* 67: 39–57. https://doi.org/10.1016/j.jeem.2013.10.002
Brauer, M., Reynolds, C. and Hystad, P. (2013). Traffic-related air pollution and health in Canada. *Can. Med. Assoc. J.* 185: 1557–1558. https://doi.org/10.1503/cmaj.121568
Burgaz, S., Demircigil, G.C., Yılmazer, M., Ertaş, N., Kemaloğlu, Y. and Burgaz, Y. (2002). Assessment of cytogenetic damage in lymphocytes and in exfoliated nasal cells of dental laboratory technicians exposed to chromium, cobalt, and nickel. *Mutat. Res*. 521: 47–56. https://doi.org/10.1016/s1383-5718(02)00215-2
Carpenter, D.O. and Bushkin-Bedient, S. (2013). Exposure to chemicals and radiation during childhood and risk for cancer later in life. *J. Adolesc. Health* 52: S21–S29. https://doi.org/10.1016/j.jadohealth.2013.01.027
Ceretti, E., Feretti, D., Viola, G.C.V., Zerbini, I., Limina, R.M., Zani, C., Capelli, M., Lamera, R., Donato, F. and Gelatti, U. (2014). DNA damage in buccal mucosa cells of pre-school children exposed to high levels of urban air pollutants. *PLoS One* 9: e96524. https://doi.org/10.1371/journal.pone.0096524
Chen, C.H., Chan, C.C., Chen, B.Y., Cheng, T.J. and Guo, Y.L. (2015). Effects of particulate air pollution and ozone on lung function in non-asthmatic children. *Environ. Res*. 137: 40–48. https://doi.org/10.1016/j.envres. 2014.11.021
Choo, C.P., Jalaludin, J., Hamedon, T.R. and Adam, N.M. (2015). Preschools' indoor air quality and respiratory health symptoms among preschoolers in Selangor. *Procedia Environ. Sci.* 30: 303–308. https://doi.org/10.1016/j.proenv.2015.10.054
Daud, S.A.M., Jalaludin, J. and Sopian, N.A. (2018). Air pollutants exposure and frequency of micronuclei (MN) among primary school children nearby industrial area. *Malaysian J. Med. Health Sci.* 14: 56–62.
Demircigil, G.C., Erdem, O., Gaga, E.O., Altuğ, H., Demirel, G., Özden, Ö., Arı, A., Örnektekin, S., Döğeroğlu, T., Doorn, W.V. and Burgaz, S. (2014). Cytogenetic biomonitoring of primary school children exposed to air pollutants: Micronuclei analysis of buccal epithelial cells. *Environ. Sci. Pollut. Res*. 21: 1197–1207. https://doi.org/10.1007/s11356-013-2001-6
Dominick, D., Juahir, H., Latif, M.T., Zain, S.M. and Aris, A.Z. (2012). Spatial assessment of air quality patterns in Malaysia using multivariate analysis. *Atmos. Environ*. 60: 172–181. https://doi.org/10.1016/j.atmosenv.2012.06.021
Donato, F. and Gelatti, U. (2014). DNA damage in buccal mucosa cells of pre-school children exposed to high levels of urban air pollutants. *PLoS One* 9: e96524. https://doi.org/10.1371/journal.pone.0096524
Elhadi, R.E., Abdullah, A.M., Abdullah, A.H., Jamalani, M. and Chng, L. (2017). A GIS-based emission inventory at 1 km-1 km spatial resolution for Particulate Matter (PM_{10}) in Klang Valley. Malaysia. *Sci. Int.* 29: 49–52.
Estrella, B., Estrella, R., Oviedo, J., Narváez, X., Reyes, M. T., Gutiérrez, M. and Naumova, E.N. (2005). Acute respiratory diseases and carboxyhemoglobin status in school children of Quito, Ecuador. *Environ. Health Perspect.* 113: 607–611. https://doi.org/10.1289/ehp.7494
Fenech, M. (2002). Chromosomal biomarkers of genomic instability relevant to cancer. *Drug Discovery Today* 7: 1128–1137. https://doi.org/10.1016/S1359-6446(02)02502-3
Fenech, M., Knasmueller, S., Bolognesi, C., Bonassi, S., Holland N., Migliore, L., Palitti, F., Natarajan, A.T. and Kirsch-Voldersh, M. (2016). Molecular mechanisms by which *in vivo* exposure to exogenous chemical genotoxic agents can lead to micronucleus formation in lymphocytes *in vivo* and *ex vivo* in humans. *Mutat. Res*. 770: 12–25. https://doi.org/10.1016/j.mrrev.2016.04.008
Han, M., Guo, Z., Li, G. and Sang, N. (2013). Nitrogen dioxide inhalation induces genotoxicity in rats. *Chemosphere* 90: 2737–2742. https://doi.org/10.1016/j.chemosphere.2012.11.057
Holland, N., Fucic, A., Merlo, D.F., Sram, R. and Kirsch-Volders, M. (2011). Micronuclei in neonates and children: Effects of environmental, genetic, demographic and disease variables. *Mutagenesis* 26: 51–56. https://doi.org/10.1093/mutage/geq064
Huen, K., Gunn, L., Duramad, P., Jeng, M., Scalf, R. and Holland, N. (2006). Application of a geographic information system to explore associations between air pollution and micronucleus frequencies in African American children and adults. *Environ. Mol. Mutagen*. 47: 236–246. https://doi.org/10.1002/em.20193
Ismail, I.N., Jalaludin, J., Bakar, S.A., Hisamuddin, N.H. and Suhaimi, N.F. (2019). Association of traffic related air pollution with DNA damage and respiratory symptoms among primary school children in Selangor. *Asian J. Atmos. Environ.* 13: 106–116. https://doi.org/10.5572/ajae.2019.13.2.106
Khan, F., Latif, M.T., Juneng, L., Amil, N., Mohd Nadzir, M.S. and Syedul Hoque, H.M. (2015). Physicochemical factors and sources of particulate matter at residential urban environment in Kuala Lumpur. *J. Air Waste Manage. Assoc.* 65: 958–69. https://doi.org/10.1080/10962247.2015.1042094
Koehler, C., Ginzkey, C., Friehs, G., Hackenberg, S., Froelich, K., Scherzed, A., Burghartz, M., Kessler, M., and Kleinsasser, N. (2011). Ex vivo toxicity of nitrogen dioxide in human nasal epithelium at the WHO defined 1-h

limit value. *Toxicol. Lett*. 207: 89–95. https://doi.org/10.1016/j.toxlet.2011.08.004

Kuwata, M., Neelam-Naganathan, G.G., Miyakawa, T., Khan, M.F., Kozan, O., Kawasaki, M., Sumin, S. and Latif, M.T. (2018). Constraining the emission of particulate matter from Indonesian Peatland Burning using continuous observation data. *J. Geophys. Res.* 123: 9828–9842. https://doi.org/10.1029/2018JD028564

Lahiri, T., Roy, S., Basu, C., Ganguly, S., Ray, M.R. and Lahiri, P. (2000). Air pollution in Calcutta elicits adverse pulmonary reaction in children. *Indian J. Med. Res*. 112: 21–26.

Latif, M.T., Dominick, D., Ahamad, F., Khan, M.F., Juneng, L., Hamzah, F M. and Nadzir, M.S.M. (2014). Long term assessment of air quality from a background station on the Malaysian Peninsula. *Sci. Total Environ.* 482–483: 336–348. https://doi.org/10.1016/j.scitotenv.2014.02.132

Mielzynska, D., Blaszczyk, E., Butkiewicz, D., Durzynska, J. and Rydzanicz, M. (2013). Influence of genetic polymorphisms on biomarkers of exposure and effects in children living in Upper Silesia. *Mutagenesis* 28: 591–599. https://doi.org/10.1093/mutage/get037

Migliore, E., Berti, G., Galassi, C., Pearce, N., Forastiere, F., Calabrese, R., Armenio, L., Biggeri, A., Bisanti, L., Bugiani, M., Cadum, E., Chellini, E., Dell'Orco, V., Giannella, G., Sestini, P., Corbo, G., Pistelli, R., Viegi, G. and Ciccone, G. (2009). Respiratory symptoms in children living near busy roads and their relationship to vehicular traffic: Results of an Italian multicenter study (SIDRIA). *Environ. Health* 8: 27–43. https://doi.org/10.1186/1476-069X-8-27

Noor Hisyam, N.H. and Juliana, J. (2014). Association between indoor PM_{10}, $PM_{2.5}$ and NO_2 with airway inflammation among preschool children at industrial and sub-urban areas. *Adv. Environ. Biol.* 8: 149–159.

Rahman, S.R.A., Ismail, S.N.S., Sahani, M., Ramli, M.F. and Latif, M.T. (2017). A case crossover analysis of primary air pollutants association on acute respiratory infection (ARI) among children in urban region of Klang valley, Malaysia. *Ann. Trop. Med. Public Health* 10: 44–55.

Rijnders, E., Janssen, N.A., van Vliet, P.H. and Brunekreef, B. (2001). Personal and outdoor nitrogen dioxide concentrations in relation to degree of urbanization and traffic density. *Environ. Health Perspect.* 109: 411–417. https://doi.org/10.1289/ehp.01109s3411

Risom, L., Moller, P. and Loft, S. (2005). Oxidative stress-induced DNA damage by particulate air pollution. *Mutat. Res.* 592: 119–137. https://doi.org/10.1016/j.mrfmmm.2005.06.012

Scandalios, J.G. (2005). Oxidative stress: Molecular perception and transduction of signals triggering antioxidant gene defenses. *Braz. J. Med. Biol. Res*. 38: 995–1014. https://doi.org/10.1590/s0100-879x2005000700003

Shuhaili, A.F.A., Ihsan, S.I. and Faris, W.F. (2013). Air pollution study of vehicles emission in high volume traffic: Selangor, Malaysia as a case study. *WSEAS Trans Syst*. 12: 67–84.

Sisenando, H.A., de Medeiros, S.R.B., Artaxo, P., Saldiva, P.H.N. and de Souza Hacon, S. (2012). Micronucleus frequency in children exposed to biomass burning in the Brazilian Legal Amazon region: A control case study. *BMC Oral Health* 12: 1–7. https://doi.org/10.1186/1472-6831-12-6

Ślęzak, M., Skalniak, A., Groszek, B., Piątkowsk, J. and Pach, D. (2014). The effect of acute carbon monoxide poisoning on micronuclei frequency and proliferation in human peripheral blood lymphocytes (case-control study). *Przegl. Lek.* 71: 463–468.

Sopian, N.A., Jalaludin, J., Mayusi, T.Z.A.T. and Latif, M.T. (2020). Increased chromosomal damage among children in proximity to an industrial zone. *Aerosol Air Qual. Res*. 20: 944–955. https://doi.org/10.4209/aaqr.2019.12.0653

Suhaimi, N.F., Jalaludin, J. and Mohd Juhari, M.A. (2020). The impact of traffic-related air pollution on lung function status and respiratory symptoms among children in Klang Valley, Malaysia. *Int. J. Environ. Health Res*. https://doi.org/10.1080/09603123.2020.1784397

Tajudin, M.A.B.A., Khan, M.F., Mahiyuddin, W.R.W., Hodd, R., Latif, M.T., Hamid, A.H., Rahman, S.A. and Sahani, M. (2019). Risk of concentrations of major air pollutants on the prevalence of cardiovascular and respiratory diseases in urbanized area of Kuala Lumpur, Malaysia. *Ecotoxicol. Environ. Saf.* 171: 290–300. https://doi.org/10.1016/j.ecoenv.2018.12.057

Wesley, A.D. and Jalaludin, J. (2015). Indoor air pollutant exposure and eosinophil cationic protein as an upper airway inflammatory biomarker among preschool children. *Procedia Environ. Sci.* 30: 297–302. https://doi.org/10.1016/j.proenv.2015.10.053

Wong, L.P., Alias, H., Aghamohammadi, N., Ghadimi, A. and Sulaiman, N.M.N. (2017). Control measures and health effects of air pollution: A survey among public transportation commuters in Malaysia. *Sustainability* 9: 1616. https://doi.org/10.3390/su9091616

Zuurbier, M., Hoek, G., Oldenwening, M., Lenters, V., Meliefste, K., Hazel, P.V.D. and Brunekreef, B. (2010). Commuters' exposure to particulate matter air pollution is affected by mode of transport, fuel type, and route. *Environ. Health Perspect.* 118: 783–789. https://doi.org/10.1289/ehp.0901622

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