

## ORIGINAL ARTICLE

# Air Pollutants Exposure and Frequency of Micronuclei (MN) among Primary School Children nearby Industrial Area

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## ABSTRACT

**Introduction:** Air pollutants that possessed genotoxic properties have the potential to induce genetic damage. Micronuclei (MN) frequency is used as an indicator for identifying potential genotoxic exposures. A comparative cross-sectional study was carried out among primary school children in a petrochemical industrial area (N=111, Kemaman) and a rural (N=65, Dungun) area in Terengganu. **Methods:** Validated questionnaires were distributed to obtain the respondents' socio-demographic data, previous exposure and reported respiratory illness. The frequency of micronuclei was assessed in collected buccal mucosa samples of children. The air monitoring was also carried out at 6 selected schools. **Results:** Results from the statistical analysis carried out showed significant differences with  $p=0.001$  for all parameters assessed between areas, which included ultrafine particles, UFP ( $z = -4.842$ ),  $PM_{2.5}$  ( $z = -10.392$ ),  $PM_{10}$  ( $z = -11.074$ ),  $NO_2$  ( $z = -11.868$ ),  $SO_2$  ( $z = -5.667$ ), relative humidity ( $z = -5.587$ ). The MN frequency was statistically significant with  $PM_{2.5}$  ( $\chi^2 = 17.78$ ,  $p=0.001$ ) and  $PM_{10}$  ( $\chi^2 = 15.429$ ,  $p=0.001$ ). The statistical analysis also showed a significant association between UFP and coughing (PR=2.965, 95% CI=1.069-8.225). The multiple logistic regression analysis showed that the main pollutants influencing MN frequencies were UFP and  $NO_2$  with UFP (PR=1.877, 95%CI= 1.174-3.002) and  $NO_2$  (PR=1.008, 95%CI= 1.001-1.015). **Conclusion:** This study demonstrated that exposure to air pollutants may increase the risk of respiratory illness and may induce MN formation among children.

**Keywords:** Air pollutants, Indoor air quality, Respiratory health, Genotoxicity, Micronuclei (MN)

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## INTRODUCTION

The rapid growth of industries has led to environmental health degradation which eventually increases the deterioration of health conditions, both acute and chronic, especially among vulnerable groups such as children, the elderly and the infirmed. It had been postulated that children are more vulnerable than adults to environmental risks due to several factors, such as children breathe in higher volumes of air compared to adults, their body systems are still developing, and they have little control over their environment (1). It is important to know that exposure to toxic air pollutants at early stages of development would lead to irreversible damage to the children.

Nowadays, there is a growing concern about the air pollutants that can exhibit genotoxic effects on the receptors, as they are able to induce cancer. Air pollutants like particulate matter, nitrogen dioxide ( $NO_2$ ), and sulfur

dioxide ( $SO_2$ ) can cause genotoxic effects to human due to their physical and chemical properties. For instance, inhalation of particulate matter which contains metal, carcinogen organic and inorganic materials can cause DNA damage, inflammation and genomic instability (2). On the other hand, toxicokinetic of  $NO_2$  depends on its behavior as an oxidative agent and free radical properties which can deplete antigen defenses (3). Generally, the chronic exposure to a complex mixture of toxic air pollutants may induce genotoxic damage such as oxidative damage and formation of DNA adducts. These DNA adducts can promote the formation of micronuclei at the cellular level.

The micronuclei are derived from acentric chromosome fragments or whole chromosomes which failed to migrate to spindle poles during anaphase. The presence of a small nucleus in the dividing cell is providing a convenient indicator for identifying potential genotoxic exposures and chromosomal instability in humans. A higher number of MN in cells is associated with a higher risk of cancer at the early stage of life (4).

This present study intended to quantify the magnitude of air pollutants exposure at school and homes of the

children who were living close to industry and those living far away from the industry. We also investigated the potential genotoxicity of air pollutants (UFP, PM<sub>2.5</sub>, PM<sub>10</sub>, NO<sub>2</sub>, and SO<sub>2</sub>) in inducing the formation of micronuclei (MN) among children. Apart from that, we also investigated the effects of air pollutants exposure on children's respiratory health as a response to acute health effects. Findings from this study would provide a baseline information for proactive measures of early prevention in tackling respiratory health problems among primary school children. The results obtained may be useful for the primary school management to outline precaution measures to control the sources of air pollution in the future.

## MATERIALS AND METHODS

### Study Background

This study is a cross-sectional study whereby indoor air monitoring of selected pollutants and evaluation of respiratory symptoms were conducted among primary school children at a petrochemical area and a rural area in Terengganu, Malaysia. 176 primary school children aged 10-11 years old were randomly recruited from six selected primary schools. The respondents from schools located within 5 km of the petrochemical area were considered as the exposed group (N=111), while the non-exposed group (N=65) were the children from schools located more than 40 km from the industrial area. The questionnaires were adapted from The American Thoracic Society and The American Pediatric Association. They comprised of seven main parts including socio-demographic background, health status, respiratory health history, tobacco smoke exposure, eating habits, indoor and outdoor environment information.

### Study area

Terengganu state was selected as the study location because of its largest and oldest petrochemical industry in Peninsular of Malaysia. The petrochemical industry in Terengganu covers two big districts, Kemaman and Dungun with 166,750 and 149,851 total populations respectively. The study location was government primary school located within 5 km from Kertih Integrated Petrochemical Complex, meanwhile, the non-exposed or comparative location was government primary school located within 100 km from the industry and are free from industrial activity, specifically Dungun district. The study location was chosen to look specifically at the effects of the emissions from the petrochemical industrial area corresponding to previous studies (5, 6).

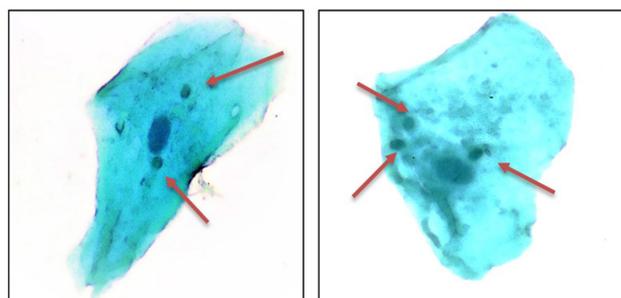
### School Monitoring

Measurements of the air pollutants and other physical parameters of IAQ at six selected schools were sampled for five hours during the schooling period. The instruments used included TSI P-Trak Ultrafine Particle Counter (UPC) 8525 for UFP, DustTrak™ II Aerosol

Monitor 8532 for PM<sub>2.5</sub> and PM<sub>10</sub>, LaMotte's Model BD Air Sampling Pump for NO<sub>2</sub> and SO<sub>2</sub>, TSI Q-Trak 7565 for temperature and humidity and TSI's Model 8386 VelociCalc for air velocity. All equipment was calibrated prior to use and were placed at 1m above the floor to avoid any obstruction.

### Collection of Exfoliated Buccal Mucosa and MN Assay

The buccal mucosa cells were taken by gently scraping both cheeks of the respondents ten times using cytology brushes. The cytology brushes containing the cells were then dipped into microcentrifuge tubes containing 0.1M Phosphate Buffer solution (pH7.5) and stored in a -20°C freezer (5). The cells were smeared onto clear glass slides followed by a fixation process with 1% glutaraldehyde in cold 0.1M phosphate buffer (pH7.5) for 20 minutes. The glass slides were stained with Schiff reagent for 1 hour and counterstained with 0.1% Fast Green for 20 seconds (5). The stained and dried slides were observed under 40x magnification of a light microscope. The results were expressed as the frequency of MN in 2000 cells (Figure 1) (7).



**Figure 1:** Arrow indicates presence of micronuclei in buccal exfoliated cell under 40x magnification

### Statistical analysis

The Statistical Package for Social Science (SPSS) Version 21.0 was utilized in tabulating and analyzing all the parameters. A normality test was performed first to assess the data distribution among the exposed and non-exposed groups. It determined the selection of subsequent statistical analysis, either parametric or non-parametric testing. Descriptive analyses were executed for the parameter of sociodemographic, air pollutants exposure and MN frequency. The median comparison of air pollutants concentration and micronuclei frequency was determined by the Mann-Whitney U test as the data were not normally distributed. Meanwhile, the respiratory symptoms were tested by Chi-Square analysis. Associations between air pollutants sources, respiratory symptoms and micronuclei frequency among children were tested by Chi-Square or Fisher's Exact test.

## RESULTS

### The concentration of air pollutants

Table 1 displays the concentration of indoor air pollutants

and IAQ physical parameter measured in the classes. Overall, the concentration of indoor air pollutants and IAQ physical parameter in classes of the exposed group was higher based on the median measured for UFP 11000 pt/cc, PM<sub>2.5</sub> 46.00 µg/m<sup>3</sup>, PM<sub>10</sub> 56.40 µg/m<sup>3</sup>, RH 69.1 %, temperature 28.7°C and velocity 0.31 m/s compared to concentration of indoor air pollutant in non-exposed group. Mann-Whitney U Test found a significant association between all indoor air pollutant concentrations and IAQ physical parameter at classes among both groups with UFP (z=-4.842, p=0.001), PM<sub>2.5</sub> (z=-10.392, p=0.001), PM<sub>10</sub> (z=-11.074, p=0.001), RH (z=-7.557, p=0.001), temperature (z=-3.954, p=0.001) and velocity (z= -5.780, p=0.001). However, the concentration of NO<sub>2</sub> and SO<sub>2</sub> measured were very low at schools of exposed group, in fact, there's no concentration detected in the non-exposed area.

### Sources of Indoor Air Pollutants

Table II shows the sources of indoor air pollutants in homes among both groups of respondents. 4 variable shows significant differences between both study group when Chi-Square was used to analyze the data. 37(33.3%) out of 111 respondents in the exposed group had parents or siblings smoking in the house, whereas 36(55.4%) in the non-exposed group had smokers in the house. The Chi-Square shows a significant difference in indoor smoke with  $\chi^2 = 8.212$ , p =0.004. Next, the Chi-Square shows a significant difference in indoor ventilation system with  $\chi^2 = 14.919$ , p =0.002, where

**Table I:** Concentration of indoor air pollutants and physical parameters of IAQ

Variable	Exposed group (n=111)	Non-exposed group (n=65)	z-value	p-value
	Median (IQR)	Median (IQR)		
UFP (pt/cc)	1100.00 (700.00)	500.00 (200.00)	-4.842	<0.001*
PM <sub>2.5</sub> (µg/m <sup>3</sup> )	46.00(20.60)	33.00(3.00)	-10.392	<0.001*
PM <sub>10</sub> (µg/m <sup>3</sup> )	56.40(11.00)	37.00(11.00)	-11.074	<0.001*
NO <sub>2</sub> (ppm)	0.28(0.43)	Lower than LOD	-11.868	<0.001*
SO <sub>2</sub> (ppm)	Lower than LOD	Lower than LOD	-	-
Relative Humidity (%)	69.10(2.20)	79.10(10.90)	-7.557	<0.001*
Temperature(°C)	28.70(0.80)	25.80(3.50)	-3.954	<0.001*
Velocity (m/s)	0.31(0.09)	0.28(0.10)	-5.780	<0.001*

N = 176, Mann-Whitney U test, \*Significant at p<0.05, LOD = limit of detection

**Table II:** Indoor air pollutant source in homes

Variable	Exposed group (n=111)	Non-exposed group (n=65)	p-value
	Total (%)	Total (%)	
Indoor home smoking			
Yes	37(33.3)	36(55.4)	8.212 <0.004*
No	74(66.7)	29(44.6)	
Ventilation system			
Fan	76(70.4)	58(89.2)	14.919 <0.002*
Air-conditioner	11(19.4)	2(3.1)	
Both fan and air-conditioner	11(10.2)	5(7.7)	
Carpets			
Yes	55(49.5)	22(33.8)	4.108 <0.043*
No	56(50.5)	43(66.2)	
Open burning			
Yes	37(33.3)	43(66.2)	17.811 <0.001*
No	74(66.7)	22(33.8)	

N=176, Chi – square test, \*Significant at p<0.05

the majority in the exposed group used the fan (70.4%) and air-conditioner (19.4%) as their ventilation system, whereas 58(89.2%) in the non-exposed group used the fan. 55(49.5%) in the exposed group and 22(33.8%) in the non-exposed group have carpets at home, which resulted in the significant difference in Chi-Square test with  $\chi^2 = 4.108$ , p =0.043. Lastly, the majority (66.2%) in the non-exposed group and 37(33.3%) in the exposed group practiced open burning at their homes. Chi-Square test shows a significant difference in open burning practice between both groups with  $\chi^2 = 17.811$ , (p <0.001). These four variables were then included in multiple linear regression which will be discussed further in their relationship in affecting the dependent variable.

### Prevalence of Respiratory Health Symptoms

Based on the statistical result shows in Table III, the respiratory symptoms were significantly higher among the exposed group (p=0.016, PR=8.490, 95%CI=1.084-4.023) for phlegm. Those who reported having cough was higher in the non-exposed group (24.6%) as compared to exposed (20.7%). Symptoms of wheezing were not common in both exposed and non-exposed (11.7% and 4.6% respectively). Besides, the symptom of chest tightness was also rarely occurred, with 2.7% in the exposed and 1.5% in the non-exposed group. Prevalence of respiratory health symptoms was then assessed from the returned questionnaires.

### Comparison of Micronuclei (MN) Frequency

Table IV displays the descriptive data micronucleus (MN) frequency found in exfoliated buccal mucosa cells of 60 children in both study groups. Normality test showed that MN frequency among respondents was not normally distributed. Thus, Mann-Whitney U test was then performed to compare the number of MN frequency between two groups study. It reveals

**Table III:** Comparison of respiratory health symptoms among respondents

Variable	Exposed group (n=111)	Non-exposed group (n=65)	$\chi^2$	p-value	PR	95% CI
	Total (%)	Total (%)				
<b>Cough</b>						
Yes	23(20.7)	16(24.6)	0.360	0.548	0.800	0.387-1.657
No	88(79.3)	49(75.4)				
<b>Phlegm</b>						
Yes	13(11.7)	1(1.5)	-	0.016*	8.490	1.084-4.023**
No	98(88.3)	64(98.5)				
<b><sup>a</sup>Wheezing</b>						
Yes	8(7.2)	3(4.6)	-	0.493	-	-
No	103(92.8)	62(95.4)				
<b><sup>c</sup>Chest tightness</b>						
Yes	3(2.7)	1(1.5)	-	1.000	-	-
No	108(97.3)	64(98.5)				

N=176, Chi-square test<sup>a</sup> Fisher-exact test for expected value <5, \*Significant at p<0.05, \*\*Significant PR at 95% CI>1

**Table IV:** Comparison of micronuclei (MN) frequency among respondents for both exposed and non-exposed group

Variable	Exposed group (n=30)	Non-exposed group (n=30)	z-value	p-value
	Median (IQR)	Median (IQR)		
MN frequency	24(14)	10(6)	-5.190	<0.001*

N = 60, Mann-Whitney U test, \*Significant at p<0.05

that there was significantly different in MN frequency between the exposed and non-exposed group with z=-5.190 and p=0.001. The genotoxicity of buccal cells was categorized as 'unlikely' (for MN frequency less than 13.5, which was the median value of MN frequency) and 'present' (for MN frequency at least 13.5). The median value of MN frequency was used as the baseline of genotoxicity as to see a variation of genotoxicity prevalence among both group study. The normality test found that MN frequency was not normally distributed, thus, Chi-square was performed to determine the difference in genotoxicity among the exposed and non-exposed group. There was a significant difference in genotoxicity among both groups, where 25(15.0%) children in the exposed group had more than 13.5 of MN frequency and only 5(16.7%) in the non-exposed group, with  $\chi^2 = 26.667$  and p=0.001. Based on Table V, there was a significant correlation between indoor air pollutants that were focused in this study and genotoxicity of buccal mucosa for UFP (r=0.533, p=0.001); PM<sub>2.5</sub> (r=0.454, p=0.001); PM<sub>10</sub> (r=0.502, p=0.001); NO<sub>2</sub> (r=-0.507, p=0.001). The correlation result of UFP, PM<sub>2.5</sub>, PM<sub>10</sub>, and NO<sub>2</sub> with r-value more than 0.3 suggested the positive correlation between these four indoor air pollutants and genotoxicity of buccal mucosa (MN frequency).

**Factors that Influenced the Genotoxicity among**

**Table V:** Correlation between indoor air pollutants with genotoxicity of buccal mucosa among the exposed group

Variables	MN Frequency (N=30)	
	r-value	p-value
UFP	0.533 <sup>c</sup>	0.001**
PM <sub>2.5</sub>	0.454 <sup>c</sup>	0.001**
PM <sub>10</sub>	0.502 <sup>c</sup>	0.001**
NO <sub>2</sub>	0.507 <sup>c</sup>	0.001**
Relative Humidity	-0.319	0.013*
Velocity	0.262	0.043*

N=30, Spearman-rho test, \*Significant at p<0.05, \*\*Significant at p<0.001,<sup>c</sup> Significant correlation at r>0.3

**Respondents**

The factors that predominantly influenced the genotoxicity of buccal mucosa were determined throughout multiple logistic regression and are tabulated in Table VI. All possible contributors were added simultaneously into the multiple logistic regressions. The results showed that there was a significant association between the indoor air pollutants of UFP (PR=1.877, 95%CI=1.174-3.002) and NO<sub>2</sub> (PR=1.008, 95%CI=1.001-1.015), and genotoxicity of buccal mucosa among the respondents from both groups.

**Table VI.** Multiple logistic regression on indoor air pollutant and MN frequency

Variables	B	S.E	p-value	OR	95% CI
UFP	0.630	0.240	0.009*	1.877	1.174-3.002**
PM <sub>2.5</sub>	0.024	0.043	0.580	1.024	0.941-1.115
PM <sub>10</sub>	-0.046	0.004	0.386	0.955	0.862-1.059
NO <sub>2</sub>	0.008	1.355	0.024*	1.008	1.001-1.015

N=60, Multiple Logistic Regression, 95% CI = Confident Interval, B = Regression Coefficient, S.E= Standard Error, OR= Adjusted Odd Ratio, \*Significant at p< 0.05, \*\*Significant OR at 95% CI >1, Nagelkerke R Square = 0.555. Adjusted for distance house from roads and industry, indoor smoking, ventilation system, carpets, open burning)

**DISCUSSION**

Generally, the air pollutants concentration in classrooms of the exposed group were significantly higher compared to the non-exposed group's. This finding is parallel to previous local studies as they found a huge significant difference of indoor air pollutants in schools nearby industrial area (5,6,9). This indicates that those residing in nearby industrial areas were exposed to higher levels of both indoor and outdoor pollutants due to the industrial emissions. These findings were consistent with many previous studies and supported the hypothesis that the location of primary schools closer to industrial areas contributed to the greater magnitude of air pollutants exposure (1, 5, 10, 11-13). Consequently, this higher exposure to indoor air pollutants at schools nearby those industrial areas might worsen the respiratory health and other related health problems.

Phlegm was the highest respiratory symptom occurred among the exposed group. A significant difference in phlegm prevalence between the two groups strongly suggest that the locality might have influenced or contributed to the occurrence of respiratory symptoms, which in the context of this study was phlegm. This would indicate that the primary school children in the exposed group were 8 times more likely to have phlegm as compared to those children in the non-exposed area. High exposure to industrial pollutants, road traffic emissions, and indoor pollutants might have contributed to the prevalence of phlegm among primary school children in the exposed group. A recent study on a petrochemical air pollutant exposure also showed greater prevalence of respiratory symptoms among primary school children with the prevalence ratio of cough, phlegm, chest tightness and wheezing at 5.09 (95% CI 2.23-11.65), 9.66 (95% CI 2.10-44.46), 9.08 (95% CI 1.09-75.0) and 9.07(95% CI 1.89-25.2) respectively (5). A larger scale study involving children living close to heavy industry in Kemaman, Malaysia also found a similar health outcome with significant associations between air pollutants exposure, in schools and homes, and respiratory symptoms (18). A study conducted in Thailand and Taiwan disclosed that people living near petrochemical industrial area experienced multiple acute respiratory symptoms from exposure to polluted air (19, 20). These indicate that proximity to industrial sites, especially petrochemical plants, plays a vital role in worsening the respiratory health of the residents nearby.

The associations between indoor UFP,  $PM_{2.5}$ ,  $PM_{10}$  and the reported respiratory symptoms inside the classrooms of the exposed group were established using the median value of indoor air pollutants measured. Based on the statistical analysis, only high levels of UFP were associated significantly with reported symptoms of cough ( $\chi^2 = 4.607$ ,  $p=0.032$ ,  $PR=2.965$ ,  $95\% CI=1.069-8.225$ ). This would elucidate why children who were exposed to the greater magnitude of UFP, were 3 times more likely to experience cough. This may be because the upper respiratory system has limited capability to eliminate UFP efficiently, thus these particles have a greater tendency to be deposited and penetrated in the lower part of the lungs (21). The unique properties of nanoparticles usually pose more serious adverse health impacts than the large sized particles (22). The scarcity of current research that focused on UFP exposure, limits the knowledge on the health effects of UFP exposure.

On the other hand, the MN frequency of children living in proximity to the industrial area was significantly higher as compared to the non-exposed group. Moreover, the MN frequency found in both groups were very high compared to the baseline MN frequency suggested for 10 to 14 years old children (MN frequency suggested = 6) based on the meta and pooled analysis (14). Apart from that, exposure to anthropogenic particulate polycyclic

aromatic hydrocarbons (PAHs) also increased the level of micronuclei in lymphocytes of children (15). To date, there is no specific cut off point of MN frequency that can be used as a reference to justify the genotoxicity level of cells. Thus, the researchers used the median of MN frequency found in both study groups to describe the possible risk of genotoxicity of buccal mucosa among the respondents in which the MN frequency has been categorized as Low (for MN frequency less than 13.5) and High (for MN frequency at 13.5 or higher). There were significant differences in genotoxicity between both groups whereby 25 (15.0%) of the children in the exposed group had more than 13.5 of MN frequency but only 5 (16.7%) in the non-exposed group ( $p<0.001$ ). Based on the statistically significant difference in the MN frequency between the two groups, the result seemed to suggest that the locality of the primary schools might have influenced the number of MN found in the respondents' cells. Furthermore, it has been extensively applied to identify potential genotoxic exposures and chromosomal instability (16). MN frequency was used in this study to look at the possible risk of genotoxicity that has a destructive effect on the genetic material of buccal mucosa cells of the children. In addition, this study exhibited higher MN frequency as compared to a study of the highly polluted province in Poland with mean micronuclei frequency  $6.56\pm 5.00$  MN per 1000 cells (15). Besides, the MN value was also higher than a study conducted at Brescia, Italy (17).

In addition, statistical analysis showed a significant association between the genotoxicity of buccal mucosa and  $PM_{2.5}$  and  $PM_{10}$  ( $p<0.001$ ). This finding was in line with a study conducted among pre-school children exposed to  $PM_{2.5}$  and  $PM_{10}$  associated with genotoxicity of buccal mucosa based on MN frequency (17). Although their results may not be directly comparable to those obtained in this study, as the ages of the children observed were different, the findings did show a significant statistical association of MN value and fine particulate exposure. Many studies have shown the genotoxicity of the fine particulate matter (17,23,24,25). Reviewed papers of the UFP potential effects on humans have also shown the potential genotoxicity of UFP (22). To date, the genotoxic effects of air pollutants are still not widely exposed which consequently limit the discussion on the related findings.

The factors that predominantly influenced the genotoxicity of buccal mucosa were determined throughout multiple logistic regression. All possible contributors were added simultaneously into the multiple logistic regressions. The results showed that there was a significant association between the indoor air pollutants of UFP ( $PR=1.877$ ,  $95\%CI=1.174-3.002$ ) and  $NO_2$  ( $PR=1.008$ ,  $95\%CI=1.001-1.015$ ), and genotoxicity of buccal mucosa among the respondents from both groups. Based on the regression analysis performed, the respondents who were exposed to UFP were over 1.9

times more likely to have genotoxicity of buccal mucosa than those who were not exposed to UFP, all other variables being the same. A reviewed paper on UFP has indicated that there could be a potential genotoxicity of UFP on human beings (21).

## CONCLUSION

This study has indicated that indoor air pollutants, especially UFP and NO<sub>2</sub>, in schools are most likely to have an impact on the micronuclei (MN) frequency found in the exfoliated buccal mucosa. Reported respiratory symptoms were consistently observed in the present exposure to air pollutants, especially UFP. Moreover, the association found between indoor air pollutants exposure and MN frequency would advocate that this biomarker can be a surrogate in evaluating the genotoxicity exposure of environmental pollutants among healthy persons. Higher reported respiratory symptoms among respondents living in industrial areas highlighted the need to improve the indoor environment and exposure prevention of poor IAQ at primary schools.

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