

Exposure to Airborne Benzene and Urinary *S*-Phenylmercapturic acid (S-PMA) Concentrations in Junior High School Students in Bandung

HONDLI PUTRA¹, BAMBANG WISPRIYONO², HARYOTO KUSNOPUTRANTO²

¹Post-Graduate Student of Public Health Study Program, Faculty of Public Health, Universitas Indonesia

²Department of Environmental Health, Faculty of Public Health, Universitas Indonesia, Kampus UI, Depok 16424, Indonesia

Correspondence to Dr. Bambang Wispriyono Email: bwispri@ui.ac.id

ABSTRACT

Background: Benzene is one of toxic substances, known as *Volatile Organic Compounds (VOC)* which has adverse impact for health such as increasing the risk of lung function impairment and leukemia on humans. The amount of benzene around the world is formed from the process of fuel combustion including motor vehicle fuel combustion. Many schools in Indonesia are located near the main road with cramped motor vehicles.

Aim: To obtain the description of benzene and *S*-phenylmercapturic Acid (S-PMA) concentrations at the school as one of benzene metabolites in students.

Methods: The measured variables were airborne benzene and S-PMA concentrations. Confounding variables were age, duration of exposure, smoking status, and type of transportation used. This is a quantitative study with cross sectional research design. This research was conducted at State Junior High School 16 of Bandung on April until May 2017. The sample was 33 people selected by the simple random sampling.

Results: Based on the result of statistical analysis at 10 spots of measurements, the airborne benzene was undetected (<0.092 ppm). The average concentration of urinary SPMA in students did not exceed the threshold limit value of 1.39 µg/g of creatinine.

Conclusion: The airborne benzene was still in low concentration with also normal concentration of S-PMA as one of the health indicator of benzene exposure.

Keywords: Benzene; *S*-phenylmercapturic Acid (S-PMA), Student, School

INTRODUCTION

Benzene (Number CAS 71-43-2; C₆H₆; molecular weight 78.1 g/mol) is one of Volatile Organic Compounds (VOC), a component of wood, fuel and tobacco smoke¹ with an aromatic compound, a single six-membered unsaturated carbon ring, colorless, volatile, highly flammable liquid with a characteristic odor and a density of 874 kg/m³ at 25°C².

In Indonesia, benzene is Hazardous and Toxic Substances (B3), it is contained in Government Regulation of the Republic of Indonesia Number 74 Year 2001³ concerning on Management of Hazardous and Toxic Substances and Threshold Limit Value of airborne benzene at work environment based on the Regulation of the Minister of Manpower and Transmigration Number 13 Year 2011 is amounted to 0.5 ppm⁴.

The result of the research conducted in Guangzhou explained a high exposure to Polycyclic Aromatic Hydrocarbons (PAH), benzene (B) and toluene (T) in children urine which was 2-30 times higher than children in developing countries⁵. A research conducted in Malaysia stated that the average of benzene was 4.6 mg/m³ indoor and 5.1 mg/m³ outdoor, and 50% from the indoor levels and 50% from outdoor levels exceeded the air quality standards for benzene of 5 µg/m³ defined by European Union⁶. In other research the result of research conducted in Tiajin China on 130 exposed workers from glue at shoe-making factory and 51 unexposed workers, a significantly higher level of S-PMA in affected sub group, 0.25 ppm higher than exposed group⁷.

World Health Organization recommended that benzene is carcinogenic for humans as category I⁸. Other research stated that exposure to air pollution could cause the decreasing of lung function in school-age children⁹. *The American Conference of Governmental Industrial Hygienist*

(ACGIH) has defined the limits of benzene exposure for workers of 0.5 ppm (1.6 mg/m³) with average exposure duration for 8 hours per day for 5 working days or 40 working hours per week¹⁰.

S-phenylmercapturic Acid is one of *biomarkers* for biological monitoring of benzene exposure in the air¹¹. In another research, *immunoassay* approach was used to analyze the S-PMA of benzene exposure level lower than 0.1 ppm¹². Schettgen developed and validated the fast, specific and very sensitive method by using LC-MS-MS-automatic multidimensional method which does not require additional sample preparation¹³. *S*-phenylmercapturic acid (S-PMA) is the result of benzene metabolite affecting the metabolism and cell development through oxidative stress¹⁴. The selection of S-PMA as urinary benzene biomarker is stated in a research that urinary benzene exposure can be detected below 1 µg/L¹². In a research conducted in Adelaide, South Australia using ELISA method on kindergarten children (4-5 years old) as its respondents, a significant relationship between children living less than 200 meters from highway and the children living more than 200 meters away was obtained¹⁵.

There was few research about air quality at school related to the students' health condition particularly in Indonesia. This research aimed to observe the health condition of junior high school students through biomarker description of S-PMA caused by benzene exposure in the school environment.

METHODS

This research is a quantitative study with cross sectional design. Cross sectional research was conducted to examine the association of exposure with disease (*disease of interest*) within a short period of time and it is able to be

used to observe the magnitude of problems and the levels of risk in a group. This research was held at State Junior High School 16 of Bandung in 2017 during April to May 2017 (the observation was conducted for 2 months). The population of this research was all the 8th grade students of State Junior High School 16 in Bandung. After calculating the number of samples, the minimum number of the samples were 33. The samples selection method in this research was simple random sampling; four people from each class of 6 classes and three people of the following 4 classes were selected from the entire 10 classes. Airborne benzene concentrations observation at the school indoor and outdoor was conducted using gas adsorption method with gas chromatography technique NIOSH Test Method 1501¹⁶. Urinary S-PMA concentrations in the sample was measured by using *liquid chromatography* tool (LC)-MS/MS⁷. Urinary creatinine was determined by Jaffe method using a creatinine kit.

RESULTS

Benzene concentrations from the measurement result showed <0.092 ppm which was the detection limit of measurement device. The minimum value of S-PMA concentration was 0.16 µg/g of creatinine and the maximum was 3.54 µg/g of creatinine. The mean value was 1.39 µg/g of creatinine with standard deviation of 1.06 µg/g of creatinine (Table 1).

The results of respondents distribution based on age variable was that grade VIII students were mainly 14 years old as many as 25 people (75.8%) with the median of age 13.8 years old. Female respondents were 20 people (60.6 %) while male respondents were 13 (39.4 %). The exposure duration for less than 8 hours per day were 29 respondents (87.9%) and the more than 8 hours were 4 respondents (12.1%). For smoking status variable, 27 respondents (81.8%) were passive smokers (smoking parents) and 6 non-smoker respondents (12.1%). The vehicle used by the students resulted 19 respondents (57.6%) riding the vehicle to go to school and 14 respondents (42.4%) were on foot (Table 2).

Based on the analysis results, they showed that there was a decrease of 0.03 µg/g of creatinine in S-PMA, from 1.43 µg/g of creatinine (exposure ≤ 8 hours) to 1.13 µg/g of creatinine (exposure > 8 hours). The result of T test was obtained the p value = 0.604, it means that there was no significant difference in S-PMA concentration statistically toward the exposure duration. S-PMA concentration increases as many as 0.51 µg/g of creatinine, from 1.07 µg/g of creatinine (non-smoker) to 1.46 µg/g of creatinine (smoker). The result of T test was obtained the p value = 0.428, it means that there was no significant difference of S-PMA concentration statistically toward smoking status. S-PMA concentration was increasing of 0.21 µg/g of creatinine, from 1.27 µg/g of creatinine (on foot) to 1.48 µg/g of creatinine (riding). The result of T test was obtained the p value = 0.577, it means that there was no significant difference of S-PMA concentration statistically toward the types of transportation used (Table 3).

Based on the analysis result of regression model after controlled by the variables was that the smoker respondents would have S-PMA level increase of 0.337.

For the respondents who ride motor vehicles, they would have S-PMA level increases of 0.596 higher than the respondents who go on foot to the school.

Table 1: Airborne benzene concentration (ppm) and urinary SPMA (µg/g of creatinine) in grade VIII students at State Junior High School 16 of Bandung, West Java Province 2017

Variable	St. Deviation	Minimum-Maximum
Benzene	1.06	0.16 – 3.54
S PMA		

Table 2: Respondent Distribution Table of grade VIII students at State Junior High School 16 of Bandung, West Java Province 2017

Variables	Total	%age
Gender		
Male	13	39.4
Female	20	60.6
Exposure duration		
< = 8 hours	29	87.9
> 8 hours	4	12.1
Smoking status		
Smoker	27	81.8
Non-Smoker	6	18.2
Types of transportation used		
On Foot	14	42.4
By Vehicle	19	57.6

Table 3: Effects of Exposure Duration, Smoking Status and Types of Transportation Used on Urinary S-PMA (µg/g of creatinine) Levels in grade VIII Students at State Junior High School 16 of Bandung, West Java Province 2017

S-PMA Levels	Mean	SD	P value
Exposure duration			
< = 8 hours	1.43	1.06	0.604
> 8 hours	1.13	1.12	
Smoking status			
Non-smoker	1.07	1.23	0.428
Smoker	1.46	1.03	
Types of transportation used			
On foot	1.27	1.13	0.577
Riding Vehicles	1.48	1.02	

DISCUSSION

The results of the study showed that airborne benzene concentration at the school environment was lower than the threshold limit value of 0.5 ppm. From the measurement results using chromatography gas NIOSH method 1501, benzene concentration was < 0.092 ppm at the entire spots of measurement. In the research conducted in Johor, Malaysia, it was obtained that VOC concentration was equal whether indoor and outdoor; however in some measurements, the outdoor concentration was higher⁶. The research conducted by Arrazy¹⁷ suggested that the average of benzene concentration in shoes worker environment was 0.345 with the range of (0-0.82 ppm). The airborne benzene concentration at the workplace was below the threshold limit value (<0.50ppm) with the average of concentration of 0.2814 ppm, while airborne benzene concentration at the workplace which was above the threshold limit value (>0.50 ppm) was 0.7539 ppm¹⁸.

The measurement result showed that the indoors air at the designated area was 0.039ppm at the minimum and

the maximum was 0.087ppm, while for non designated area was 0.042ppm at minimum and the maximum was 0.103ppm¹⁹. The research conducted in Adelaide, Australia obtained the average concentration of benzene in the air was 1.62ppb on sunny days and 1.36 ppb on windy days¹⁵.

The threshold limit value for ambient air still refers to Threshold Limit Value (TLV) of the air at the workplace based on the Regulation of the Minister of Manpower and Transmigration Number PER-13/X/2011 of 0.5 ppm. Based on the statistical test result, the average concentration of urinary S-PMA was 1.39 µg/g of creatinine with the standard deviation of 1.06 µg/g of creatinine which did not exceed the threshold limit value for workers of 25 µg/g of creatinine¹⁰. It is in accordance with low benzene concentration in the air < 0.092 ppm for benzene concentration in the air space of a school.

The result of the research conducted at a preschool children in Adelaide, Australia showed S-PMA 2.97 µmol/mol of creatinine level¹⁵. As the comparison, S-PMA concentration of Wulandari's research²⁰ on 64 informal workers in shoes industry showed an average level of S-PMA 24.627 µg/g of creatinine and exceeded 25 µg/g of creatinine of 31.3%. The research in the South of Italy involving samples of 155 children for Milazzo and 58 children in Nizza compared to the children living near the oil refinery area (Milazzo) with the children living far from the oil refinery (Nizza). The result of the research indicated that S-PMA concentration 0.20 mg/g of creatinine in the afternoon and 0.15 mg/g of creatinine in the morning (Milazzo) were compared to 0.17 mg/g of creatinine in the afternoon and 0.14 mg/g of creatinine in the morning (Nizza). This result showed that S-PMA concentration in children living near the oil refinery was higher than the children living far from the oil refinery¹⁴. The research of Wulandari²⁰ suggested that there was a significant difference of the average urinary SPMA level in workers working for >8 hours per day with the workers working for ≤ 8 hours per day.

Based on the analysis result, it showed that there was no significant difference of S-PMA concentration on the exposure duration. The measurement of benzene exposure on the research of Bahrami¹⁵ obtained a significant relationship between the exposure duration and the urinary SPMA, by the result of measurement 1.56 and 4.67 µmol/mol of creatinine with $p < 0.005$. The study conducted in Amsterdam obtained S-PMA concentration < 0.5-235 µmol/mol of creatinine in the early working hours and < 0.5-378 µmol/mol of creatinine after the working hours in the workers of natural gas production plant²¹.

Based on the analysis results, it showed that the S-PMA concentration increased as many as 0.51 µg/g of creatinine in the smokers even though statistically there was no significant difference of S-PMA concentration on the smoking status. A study conducted involving 114 Traffic Police Officers in Bologna, Italy resulted that S-PMA concentration in non-smokers was 1.12 ± 0.77 µg/g-¹ of creatinine (n=79), moderate smokers was 1.54 ± 0.69 µg/g-¹ of creatinine (n=15) and heavy smokers 1.44 ± 0.68 µg/g-¹ of creatinine (n=11). Bahrami¹⁵ suggested that there was no relationship between SPMA and smoking status of parents of students.

Researches in some countries revealed the tendency of urinary SPMA levels ranged from 5.3 up to 5.8 µg/L in non-smoking workers and 7.5 to 9.3 µg/L in smoking workers²². In addition, two researches on human showed that 50% benzene were inhaled and permeated by the body. Cigarette smoke was one of the sources of benzene exposure; benzene concentration in the blood of 14 smokers were significantly higher (median 493 ng/l) than 13 non-smokers (median 190 ng/l)².

Based on the statistical test results, it was known that SPMA levels of the respondents who go on foot to the school were lower than those riding the motor vehicles. It is in line with Van Wijnen, 1995²³ that pedestrians and bicycle riders were lower in benzene, toluene and xylene exposure compared to the people riding the motor vehicles

CONCLUSION

The airborne benzene was still in low concentration with also normal concentration of S-PMA as one of the health indicator of benzene exposure. For further research, it is suggested to examine other air pollutants for instance CO₂ and other VOC as well as to observe the changes of student's health through oxidative stress biomarker like Malondialdehyde (MDA), Glutathione (GSH) and others.

REFERENCES

1. Falzone, L. et al., 2016. Occupational exposure to carcinogens: Benzene, pesticides and fibers (Review). *Molecular Medicine Reports*, pp.4467–4474.
2. WHO, 2010. Exposure to Benzene: A Major Public Health Concern. *Disease, Preventing Healthy, Through*, p.5.
3. Peraturan Pemerintah Republik Indonesia Nomor 74 Tahun 2001 Tentang Pengelolaan Bahan Berbahaya dan Beracun.
4. Peraturan Menteri Tenaga Kerja dan Transmigrasi Nomor Per.13/Men/X/2011 Tahun 2011 tentang Nilai Ambang Batas Faktor Fisik dan Faktor Kimia di Tempat Kerja
5. Li, J. et al., 2015. Co-exposure to polycyclic aromatic hydrocarbons, benzene and toluene and their dose-effects on oxidative stress damage in kindergarten-aged children in Guangzhou, China. *Science of the Total Environment*, 524–525, pp.74–80. Available at: <http://dx.doi.org/10.1016/j.scitotenv.2015.04.020>.
6. Norbäck, D. et al., 2017. Volatile organic compounds (VOC), formaldehyde and nitrogen dioxide (NO₂) in schools in Johor Bahru, Malaysia: Associations with rhinitis, ocular, throat and dermal symptoms, headache and fatigue. *Science of The Total Environment*, 592, pp.153–160. Available at: <http://linkinghub.elsevier.com/retrieve/pii/S0048969717304734>.
7. Zhang, X. et al., 2014. Simultaneous determination of five mercapturic acid derived from volatile organic compounds in human urine by LC-MS/MS and its application to relationship study. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, 967, pp.102–109. Available at: <http://dx.doi.org/10.1016/j.jchromb.2014.07.013>.
8. IARC. 2007. Agents reviewed by the IARC monographs. Volumes 1-96. (alphabetical order). Lyon, France: International Agency for Research on Cancer. <http://monographs.iarc.fr/ENG/Classification/Listagentsalphorder.pdf>. May 1, 2007.
9. Gehring, U. et al., 2013. Air pollution exposure and lung function in children: The ESCAPE project. *Environmental Health Perspectives*, 121(11–12), pp.1357–1364.
10. American Conference Governmental Industrial Hygienists (ACGIH). 2012. Threshold Limit Value for Chemical

- Substances and Physical Agents and Biological Exposure Indices. Cincinnati, Ohio, USA.
- Melikian, A.A. et al., 2002. Personal exposure to different levels of benzene and its relationships to the urinary metabolites S-phenylmercapturic acid and trans,trans-muconic acid. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, 778(1–2), pp.211–221.
 - Farmer, P.B. et al., 2005. The use of S-phenylmercapturic acid as a biomarker in molecular epidemiology studies of benzene. *Chemico-Biological Interactions*, 153–154, pp.97–102.
 - Schettgen, T. et al., 2008. Fast determination of urinary S-phenylmercapturic acid (S-PMA) and S-benzylmercapturic acid (S-BMA) by column-switching liquid chromatography-tandem mass spectrometry. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, 863(2), pp.283–292.
 - Andreoli, R. et al., 2015. Urinary biomarkers of exposure and of oxidative damage in children exposed to low airborne concentrations of benzene. *Environmental Research*, 142, pp.264–272. Available at: <http://dx.doi.org/10.1016/j.envres.2015.07.003>.
 - Bahrami, A.R, Edwar, J.W. 2006. Evaluation of benzene exposure in adult and urinary s-phenylmercapturic acid in living in adelaide, South Australia. *International Journal of Environment Science and Technology*. Vol. 3, Num 2, 2006, pp. 113-117.
 - NIOSH, 2003. Hydrocarbons, Aromatic: Method 1501. NIOSH Manual of Analytical. Methods. 4th Edition. <https://www.cdc.gov/niosh/docs/2003-154/pdfs/1501.pdf>
 - Arrazy, S., 2016. Analisis Hubungan S-Phenylmercapturic Acid Sebagai Metabolit Benzene dengan Kadar Malondialdehyde Pada Pekerja Pabrik Sepatu Di Sentra Industri Sepatu Cibaduyut Tahun 2016. [Tesis]. Fakultas Kesehatan Masyarakat Universitas Indonesia.
 - Sanjaya, B. R., 2016. Asosiasi Paparan Benzene Terhadap Kadar Hemoglobin (Studi pada Pekerja Laki-Laki di Industri Sepatu Informal Cibaduyut Jawa Barat. [Tesis]. Fakultas Kesehatan Masyarakat Universitas Indonesia.
 - Kesehatan, 2014. Gambaran Paparan Benzene Dalam Rumah Terhadap Profil Darah Kawasan Industri dan Pemukiman. Preview of Benzene Indoors and Blood Profile in The Industrial Zone and Community.
 - Wulandari, P. et al., 2017. Urinary S-Phenylmercapturic Acid (S-PMA) Level as Biomarkers of Exposure to Benzene in Informal Shoes Industrial Workers, Cibaduyut Bandung. *ICGH Conference Proceedings. The 1st International Conference on Global Health*. KnE Life Sciences. pages 84-92. DOI 10.18502/kls.v4i1.1369.
 - Boogaard, P.J. & Van Sittert, N.J., 1996. Suitability of S-phenyl mercapturic acid and trans-trans-muconic acid as biomarkers for exposure to low concentrations of benzene. *Environmental Health Perspectives*, 104(Suppl. 6), pp.1151–1157.
 - Fustinoni, S. et al., 2005. Urinary t,t-muconic acid, S-phenylmercapturic acid and benzene as biomarkers of low benzene exposure. *Chemico-Biological Interactions*, 153–154, pp.253–256.
 - Van Wijnen JH. et al., 1995. The exposure of cyclists, car drivers and pedestrians to traffic-related air pollutants. *Int Arch Occup Environ Health*.67(3):187-93.