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Urinary S-Phenylmercapturic Acid (S-PMA) Level and Leukocytes in Informal Shoes Industrial Workers Exposed to Benzene in Cibaduyut, Bandung Area

Bambang Wispriyono, Puri Wulandari, Syafran Arrazy, Bayu Rizki,
Haryoto Kusnoputranto and Laila Fitria
Department of Environmental Health, Faculty of Public Health,
Universitas Indonesia, Depok, Indonesia

Abstract: Benzene exposure in working environment has been known associated to hematology disorders. This is because the hematology system is the most critical tissue in benzene exposure through inhalating route. This study aims to analyze the relation between urinary S-PMA level and number of leukocytes of informal shoes industrial workers exposed to benzene. This study used cross sectional design in six informal shoes industries which are located in Cibaduyut with the number of sample of 64 workers. Urinary and blood samples were collected on each sample to measure urinary S-PMA level and the number of leukocytes. Urinary S-PMA level was measured by using LC-MS/MS and leucocytes was measured by using automated hematology analyzer. Individual characteristic data were obtained through direct interview and benzene concentration in environment was collected from secondary data. The result of the study indicated that there is significant correlation between urinary S-PMA level with leukocytes (p-value: 0.048) and urinary S-PMA level with the type of job (p-value: 0.004). By 31.3% workers have urinary S-PMA level more than Biological Exposure Indices-American Conference of Governmental Industrial Hygienist (BEI-ACGIH) (>25 μg/g creatinine). The higher the benzene concentration of indoor air, the more workers have urinary S-PMA level >25 μg/g creatinine. The result of double linear regression test finds that there is association tendency between urinary and leukocytes S-PMA level, after it is controlled by type of job, time of work per day and exercising habit variables. It can be concluded that there is association between urinary S-PMA level and the number of leukocytes decreased in the workers.

Key words: Benzene, S-phenylmercapturid acid, leukocytes, informal workers, shoes industries, S-PMA

INTRODUCTION

Benzene as one of Volatile Organic Compounds (VOC), since, the middle of 19th century has been widely used in rubber industry as the solvent for glue component (Hodgson, 2004). It is about 14.8 million ton benzene/year released to the environment (Anonymous, 2007a). The ability to dissolve insoluble substance in water is very interesting for producer party, so, it brings logical consequence of benzene concentration increase in the air (Hodgson, 2004). Today, the use of benzene has been estimated to reach eleven millions gallon each year (Hodgson, 2004) as solvent material of various kinds of industry, those are chemical industry, shoes industry, paint solvent, components in detergent, pesticide and pharmacy industry (Arnold *et al*., 2013; Anonymous, 2007, 2013). Benzene is also be found in air pollutant as well as in forest/woods firing emission, charcoal burning, motor vehicle emission, gasoline fumes and exposure of cigarettes smoke (Anonymous, 2013; 2007b; Minciullo *et al*., 2014; Rappaport *et al*., 2009). Benzene is also a problem, especially on oil industry because benzene is one of components of petroleum as well as the wide use of benzene derivatives (toluene and xylene) in various kinds of industry (Minciullo *et al*., 2014). The use of benzene as solvent in shoes industry both in developed countries and developing countries has been limited. This is due to benzene has carcinogens effect for human based on Anonymous (2012a, b) and Richardson (2008). However, benzene exposure with high concentration is assumed to remain occur in developed and developing countries. In developing countries, benzene is still used in many industries, although, some countries has restricted the use of benzene (Wang *et al*., 2012a, b; Anonymous, 2007). The use of chemical materials like organic solvent today has widely developed and free along with rapid industrial development including informal industrial sector. One of informal industrial sector that uses organic solvent in production process is shoes industry that uses glue material. In Indonesia, benzene is

Corresponding Author: Bambang Wispriyono, Department of Environmental Health, Faculty of Public Health, Universitas Indonesia, Depok, Indonesia

still also be used in shoes industry, printing factory, furniture industry and so on, although, benzene has been classified as hazardous material in Indonesian Government regulation.

ACGIH has standard for benzene is 0.5 ppm (1.6 mg/m^3) for Time Weighted Average (TWA) and 2.5 ppm (8 mg/m^3) for Short Term Exposure Limit (STEL) (Anonymous, 2007). Occupational Safety and Health Administration (OSHA) permitted benzene exposure 1 ppm (5 mg/m^3) for 8 h exposure and 5 ppm (15 mg/m^3) for 15 min exposure (Anonymous, 2012). In Indonesia based on Ministry of Manpower and Transmigration RI's Regulation No. 13/MEN/X/2011, benzene has been classified in A1 group with TLV TWA 0.5 ppm and STEL 2.5 ppm. Base on Standar Nasional Indonesia, benzene has been classified in A2 group with TLV TWA 10 ppm or 32 mg/m^3 benzene in air (Anonymous, 2005). Benzene high exposure with high and low concentration in shoes industry is always correlated with hematology disorders to workers (Wang *et al*., 2012; Kim *et al*., 2005, 2006; Lan *et al*., 2004). This is caused by hematology system is the most critical target toward benzene exposure through inhaling route, especially on bone marrow part. It results in disorders in thrombosis process which is the decrease of main blood components, either erythrocytes, leukocytes, thrombocytes or the three of them (pancytopenia) (Arnold *et al*., 2013; Lan *et al*., 2004; Qu *et al*., 2002; Ibrahim *et al*., 2014). However, in some cases there is also bone marrow permanent breakdown which can cause aplastic anemia and leukemia (Hodgson, 2004). Benzene Acute REL (Reference Exposure Level) 27 μg/m^3 (0.008 ppm; 8 ppb) has critical effects in developmental hematotoxicity in fetal and neonatal mice with Hazard Index targets in developmental, immune system and hematologic system. Benzene chronic REL 3 μg/m^3 (0.001 ppm; 1 ppb) has critical effects in decreased peripheral blood cells with hazard Index targets in hematologic system (Collins, 2014). Since, the carcinogenicity effect, Anonymous (2009) has not permitted the using of benzene in any industries.

The use of biomarker as biological exposure marking has an advantage to show the relation between environmental exposure and disease risk. Study measuring exposure with biomarker is important to be conducted because biomarker can represent exposure of various lines occurrence and describe the exposure intensity during certain time interval (Arnold *et al*., 2013). S-PMA is produced from condensation of bezene oxide with glutathione. It is one of benzene metabolites in the body as well as t, t-muconic Acid (ttMA), epoxybenzenediol, phenol, benzoquinone an hydroxybenzene as shown in Fig. 1 (Arnold *et al*., 2013). S-PMA has been choosen for benzene exposure, since, it is the most sensitive and specific for benzene exposure compare to other metabolites like tt-MA that can be also produced from any agents beside benzene like toluene, xylene or sorbic acid from food products (Lv *et al*., 2014; Qu *et al*., 2002; Hoet *et al*., 2009). S-PMA has half-life 9-13 h and for the second phase slower with half life 45 h (Sittert *et al*., 1993; Arnold *et al*., 2013).

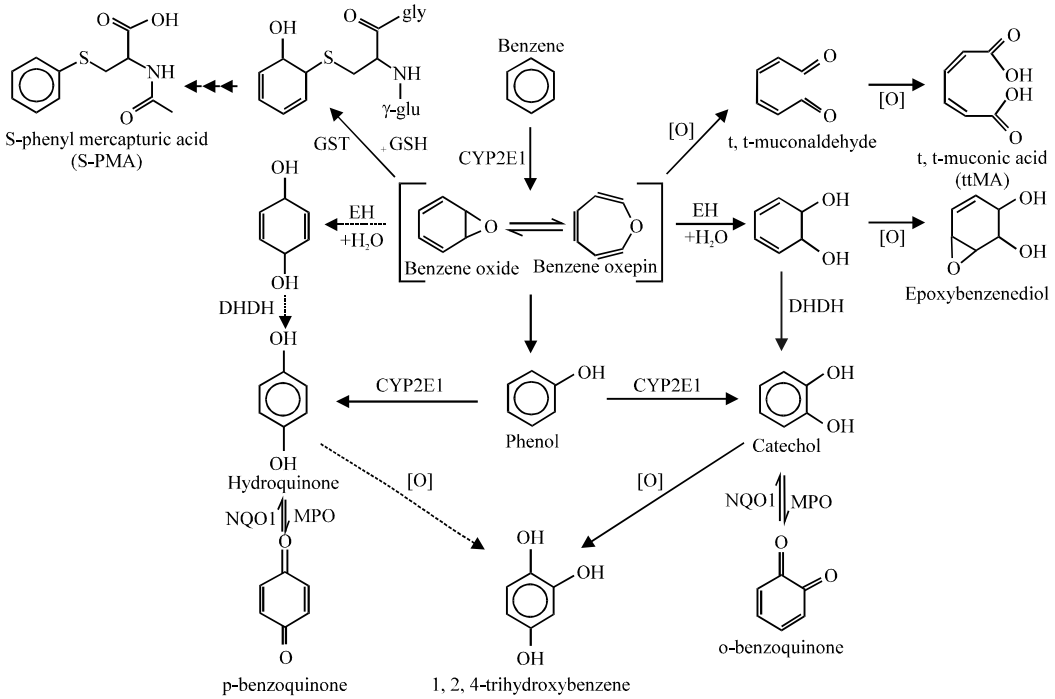

Fig. 1: Metabolism of benzene in human body (Arnold *et al*. 2013)

Leucocytes also has been choosen for benzene exposure, since, leucocyte is the early impact in hematology system for benzene-exposed workers in shoe-making industries. (Qu *et al*., 2002; Lan *et al*., 2004; Schnatter *et al*., 2010; Avogbe *et al*., 2011). In Indonesia, there are many shoe making industries and they use glue/adhesive-containing benzene. Since, they are mostly home industries or small factory, many workers work without body protective equipments and without strictly inspection. As the consequence, many workers have potential risk from benzene exposure (Wang *et al*., 2006, 2012; Kim *et al*., 2005, 2006). The aim of the study is to analyze the relation between urinary S-PMA and leukocytes as biomarkers of benzene exposure of informal shoes industrial workers.

MATERIALS AND METHODS

This study used cross sectional design aiming at measuring the level of exposure which is urinary S-PMA level with leukocytes as dependent variable. Sampling has been done for 2 months in April-June 2016. Population in this was all informal shoes industry workers working at Cibaduyut area which is located in Bandung city and regency. This study was conducted at randomized six industries from more than 30 shoes industry located in two areas which are Bandung city (Cibaduyut Wetan and Cibaduyut Kidul urban villages) and Bandung District (Cangkuang Kulon and Sukamenak villages). Study samples were then selected based on inclusion and exclusion criteria.

Respondents were choosen by the inclusion criteria: having employees more than 10 people has been more than 1 year production having production rooms; male employees; Age >18 years old; in location during research period and agree to involve in the research. We choosed 6 home industries randomize from two district in Bandung. From every disctrict, we chooseed 3 home factory industries and got total 90 workers. We got 68 respondents with inclusion criteria and after taking the urine and measuring the urine creatinine in the lab, we made another inclusion for respondents should be in between 30-300 mg/dL as the normal urine creatinine for monitoring biology (Anonymous, 1996) and final respondents were 64 respondents.

Individual characteristic data were collected by interview method using questionnaire with consist of question in characteristics, working history, healthy behavior, infection history and sport activity. We used questionnaire from previous doctoral research of Faculty of Public Health Universitsa Indonesia (Not published Fitria *et al*.). Benzene concentration data in indoor air were collected from environmental monitoring's secondary data. Worker's urinary samples were collected once which was in the end of working shift. Urinary sample volume was collected minimum by 10 mL per worker and was conducted by the workers themselves. Urine samples were treated by referrint to the study by Lv *et al*. (2014). S-PMA level in urinary sample was measured using LC-MS/MS instrument with QC (Arnold *et al*., 2013). Each worker who was observed would be collected the blood by 3 cc of blood sample (vein blood of inside part/ the folds of elbow). Blood samples were collected in same date with urinary sample collection and was performed by laboratory officer and then was analyzed using automated hematology analyzer (Ibrahim *et al*., 2014). Urine samples were kept in -4°C for one day before further analysis and blood samples directly were analysis after collecting the samples.

Variables observed in this study is S-PMA level in urinary, leukocytes, benzene concentration in air, workers characteristic involving age, working time, working hours, smoking status, alcohol intake, infection history, exercising habit and type of job. Data processing used SPSS 18.0 Software and data analysis used correlation, linear regression, t-test, ANOVA test and double linear regression tests. This study had passed ethical study procedure and was declared eligible to be conducted by Committee of Experts for Research and Ethics Research, Faculty of Public Health Universitas Indonesia on 1st June 2016 with the number: 170/UN2.F10/PPM.00.02/2016.

RESULTS AND DISCUSSION

Total respondents were 64 (response rate = 85%). Respondents with inclusion criteria and after excluding from urine creatinine measurement, we got 60 respondent. The characteristics of respondents is shown in Table 1.

Table 1: Distribution of respondents

Characteristics	Number (n)	Percentage
Education		
Not pass element school	3	4.7
Elementary school	23	35.9
Junior hight school	29	45.3
Senior high school	9	14.1
Type of work		
Gluing	36	78.1
Non gluing	28	43.8
Work hours per day		
>8 h	53	82.8
≤8 h	11	17.2
Smoking status		
Light (0-200)	46	71.9
Moderate (201-600)	16	25.0
Heavy (>600)	2	3.1
Smoking at work (n:50)		
Yes	43	86.0
No	7	14.0
Alcohol consumption		
Yes	11	17.2
No	53	82.8
Infection history in 1 the last 1 month		
Yes	3	4.7
No	61	95.3
Exercise in a week		
No	18	28.1
Yes	46	71.9

Table 2: Distribution S-PMA level in urinary according to the benzene air concentrations in the workplace informal shoes industrial in Cibaduyut, Bandung area

		S-PMA level*)				
		>25 μg/g creatinine (high exposed)		≤25 μg/g creatinine (low exposed)		
Industry	Benzene concentration (mg/m^3)	n	Percentage	n	Percentage	Total n
A	0.6867	4	80.0	1	20.0	5
B	0.3381	2	33.3	4	66.7	6
C	nd	1	7.7	12	92.3	13
D	0.8211	12	44.4	15	55.6	27
E	nd	1	11.1	8	88.9	9
F	0.2247	0	0	4	100.0	4

*)25 μg/g S-PMA creatinine is BEI ACGIH value for occupationally exposed; nd = not detecable

Table 3: Distribution S-PMA level in urinary and leukocytes of informal shoes industrial workers in Cibaduyut, Bandung area

Variables	Mean	Median	SD	Min-Max	95% CI	n (%)
S-PMA level (μg/g creatinine)	24.62	10.24	39.67	0.18-211.82	14.71-34.53	
>25 μg/g creatinine		-	-	-	-	20 (31.3)
≤25 μg/g creatinine		-	-	-	-	44 (68.8)
Leukocytes (×103/μL)	7.60	7.45	1.69	5.10-15.80	7.18-8.03	

Table 4: Distribution S-PMA level in urinary according to smoking status, alcohol consumption, working hours per day and type of job in informal shoes industrial workers in Cibaduyut, Bandung area

Variables	N	Mean	SD	SE	p-values
Smoking status					
Light	46	0.881	0.838	0.123	0.834
Moderate	16	0.911	0.674	0.168	
Heavy	2	0.548	0.954	0.675	
Alcohol consumption					
Yes	11	1.065	0.783	0.236	0.395
No	53	0.839	0.796	0.109	
Working hours per day					
>8 h	53	0.859	0.775	0.106	0.673
≤8 h	11	0.971	0.906	0.273	
Type of job					
Glueing	36	1.126	0.713	0.119	0.004
Non glueing	28	0.559	0.787	0.148	

Benzene concentration has been measured in six industrial location (A-F). In higher benzene concentration (A and D industries), the workers's S-PMA levels higher than 25 mg/g creatinine as the cut off group for higher benzene exposed and low benzende exposed (Table 2).

All total 64 sampling respondents have been included. In Table 3, it is shown that 31.3% of the workers have S-PMA level higher than 25 μg/g creatinine, however, the mean leukocyte of them are still in the normal range (7.60×10^3/μL).

There is no statistically difference between the levels of S-PMA urine with smoking status, alcohol consumption and working hours per day ($p>0.05$). However, there is a difference between the levels of S-PMA urine (p-value: 0.004) between the glueing's workers and non glueing's workers as shown in Table 4. Non glueing means the workers worked in activities with low exposed's benzene like disaigning, making and cutting pattern stitching material, sewing material, finishing/packaging unit and administration unit.

Table 5: Correlation and regression analysis of S-PMA level in urinary, age and length of work with length of work dengan leukocytes of informal shoes industrial workers exposed to benzene in Cibaduyut, Bandung area

Variables	r	R^2	Regression equation	p-values
S-PMA level	-0.248	0.062	$Y = 8.074-0.531X_1$	0.048
Age	0.034	0.001	$Y = 7.440+0.005X_2$	0.787
Length of work	0.042	0.002	$Y = 7.533+0.006X_3$	0.743

Y: Leukocytes; X_1: S-PMA level; X_2: Age; X_3: Length of work

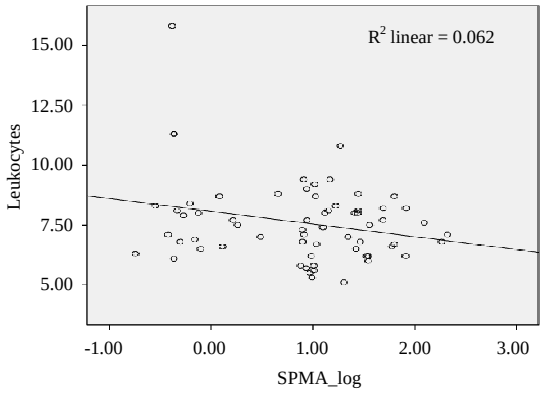

Fig. 2: Regression line equation graph level of S-PMA in urine and leukocyte workers

The number of leukocytes has statistically significant correlation with the levels of S-PMA urine with ($p<0.05$). However, there is no significant correlation between age and length of work with leucocytes ($p>0.05$). S-PMA urine levels, age and length of employment affects the number of leukocytes 6.2, 0.1 and 0.2%, respectively (Table 5).

Based on the chart in Fig. 2, it shows that the higher the level of S-PMA in the urine, the lower the number of leukocytes at workers and there is no statistically difference in mean leukocyte with smoking

Table 6: Distribution of leukocyte according smoking status, alcohol consumption, history of infections, exercising habit, working hours per day and type of job in informal shoes industrial workers in Cibaduyut, Bandung area

Variables	N	Mean	SD	SE	p-values
Smoking status					
Light	46	7.45	1.86	0.27	0.487
Moderate	16	8.00	1.19	0.29	
Heavy	2	8.15	0.21	0.15	
Alcohol consumption					
Yes	11	7.47	1.81	0.54	0.774
No	53	7.63	1.69	0.23	
History of infection					
Yes	3	6.26	0.25	0.14	0.162
No	61	7.67	1.71	0.21	
Exercising habit					
Non a routine	18	7.87	2.40	0.56	0.430
Routine	46	7.50	1.34	0.19	
Working hours per day					
>8 h	53	7.76	1.78	0.24	0.110
≤8 h	11	6.86	0.87	0.26	
Type of job					
Glueing	36	7.58	1.42	0.24	0.897
Non glueing	28	7.64	2.02	0.38	

status, alcohol consumption, history of infection, exercise habits, hours of work per day and the type of work ($p>0.05$) as shown in Table 6.

Multivariate analysis: Multivariate analysis is conducted by linear regression method to analyze the correlation between S-PMA level and the number of worker's leukocytes controlled by age, length of work (year), working hours, smoker status, alcohol intake, infection history, exercising habit and type of job variables. Because S-PMA level data and working time data are not normally distributed, then in this analysis, S-PMA level variable is used which is transformed with log 10 while variable of working time is transformed by squared method (square). The initial model is implemented toward all variables without considering p-value with the consideration that substantially, all variables are risk factors for the number of leukocytes. Linear regression can be seen on Table 7.

In the model, it can be stated that any increase in the levels of S-PMA at 1 μg/g creatinine can reduce the number of leukocytes by $0.546\times10^3/\mu L$ after it is controlled by type of job, working hours per day and exercising habit variables. The regression equation: y(leukocytes) = 9.882-0.546 (S-PMA)-0.195 (type of job)-0.330 (exercising habit)-0.809 (working hours per day).

The average of urinary S-PMA level is still below Biological Exposure Indices (BEIs) ACGIH value which is by 25 μg/g creatinine. BEIs ACGIH number is the reference number for standar in Indonesia law. Based on the value, then workers proportion with S-PMA value >25 μg/g creatinine by 31.3%. This is caused by benzene

Table 7: The final model of multivariate analysis with linear regression of S-PMA level in urinary informal shoes industrial workers in Cibaduyut, Bandung area

Variables	$Coef_{adjusted}$	p-values	-------95% CI -------	
SPMA level	-0.546	0.059	-1.113	0.021
Type of job	-0.195	0.666	-1.097	0.706
Exercising habit	-0.330	0.477	-1.252	0.592
Working hours per day	-0.809	0.150	-1.917	0.300

Constant, 9.882

concentration in indoor air in six industries are still in normal limit. This is different with the study result conducted toward shoes industrial workers in China which indicates that workers exposed to benzene with its concentration in air by = 6.0; 6.0-10.0; 10-32.5 mg/m^3 have average of urinary S-PMA level of 49.55; 102.15 and 335.69 μg/g creatinine, respectively (Lv *et al*., 2014). This is due to benzene concentration measured exceeds quality standard with continuous exposure. Urinary metabolite measurement (S-PMA, t, t-MA, phenol, hydroquinone, catechol) is extremely at risk of compound lost due to evaporation process and possibility of contamination, however we did not measure the t, t-MA or other metabolite, since, the limitation of research funding and S-PMA is more sensitive metabolite compare to others. We choosed S-PMA and not measure tt-MA, since, the limitation of research funding and we also have done research on tt-MA in gasoline pump's workers in Depok city, West Java in 2005 which is the result was no significant (data not published). This is different when using benzene metabolite in blood which does not consider compound lost due to evaporation process during blood sampling compares to use metabolite in urinary (Arnold *et al*., 2013). However, when it is compared to blood metabolite measurement, urinary metabolite (S-PMA) has strength of after one day of work, it generally portrays the average of S-PMA level in plasma during some periods of urinary accumulation in the kidney on that day (Miraglia *et al*., 2014).

Benzene exposure in the air can be produced from cigarettes smoke. Based on this study, there is no significant different on average of S-PMA level of workers with light, moderate and heavy smoker status. It is may be because most workers are in light and moderate smokers with the number of cigarette lower than 2 cigarettes per day. On the other hand, benzene concentration which is inhaled by smokers is 10-20 times higher than non-smokers (Gordon *et al*., 2002). Smoking habit also can affect S-PMA level which is able to reach five times higher than non-smokers (Fustinoni *et al*., 2005).

Another factor affecting S-PMA level is alcohol intake. From the study result, there is no significant different of average of worker's S-PMA level who consume alcohol and not in the last 1 year. This is caused

by workers do not consume alcohol regularly like smoking habit which is performed every day. Most workers who consume alcohol admit that they only consume alcohol occasionally and on uncertain time. In average, workers consume alcohol by 1 glass. Although, alcohol intake is not performed like smoking behavior but alcohol intake can increase S-PMA level, although, it is only occasional alcohol consumption but in no small amount of alcohol (Carrieri *et al*., 2012). Alcoholic drink contains ethanol. In the body, ethanol intake and benzene exposure cause cytochrome P-450 (CYP2E1) liver isoenzymes formation, so, ethanol can increase benzene metabolism, it results in benzene metabolite formed (including S-PMA) becomes lot more. In addition, ethanol can increase benzene toxicity by increasing formation rates of benzene metabolite to be more toxic (Anonymous, 2007; Wilbur *et al*., 2008).

S-PMA level in workers urinary obtained from this study only gives exposure overview in the time when the study is conducted and give no benzene exposure accumulation overview in years. Based on the analysis there is no significant difference of the average of S-PMA level in urinary with working hours. The result is not significant because there are two industries of which concentration of benzene in indoor air are not detectable. It is assumed that glue which is used no longer contains benzene at the spot measurement. However, S-PMA concentration is varied between workers, since, they have been exposed daily. Based on this study, other studies indicate that workers exposed to low concentrate benzene by 3.25 mg/m^3 (1 ppm) for 8 h have average of urinary S-PMA level by 46 μg/g creatinine (95% CI: 41-50 μg/g creatinine) while workers exposed to high concentrate benzene by 32.5 mg/m^3 (10 ppm) for 8 working hours have urinary S-PMA level by 383 μg/g creatinine. Most workers (82.8%) work >8 h long with the average of 11 h per day. This causes urinary sampling on workers is not performed in the end of working shift but only in the afternoon considering that most workers start working on 07.00 A.M based on ACGIH, the time of urinary sampling is right after work in a day (end of shift sample) (Miraglia *et al*., 2014). The length of working hours depends of shoes orders. The more the orders, the longer the working hours is due to wholesale working system.

There is significant difference between the average of S-PMA level in urinary of workers in gluing section and non-gluing section. This indicates that benzene exposure more intensively occurs on gluing workers compares to non-gluing workers. This is caused by the work uses more shoes glue. Other factors as well as ventilation, working space and working shifting may be affected workers in gluing section inhaled more benzene's contain air the the workplace rather workers in non-gluing section. The working areas are between 32-180 m^2 with 1-3 ventilations with one fan in the room. However, we did not measure the temperature and humidity of working area. The total shoes produce in a day vary between factory to factory depend on ordering system. The glue uses in a day about 5-10 kg glue a day.

In this study, the average number of leukocytes of all workers by 7.60×10^3/μL is obtained. The average number of worker's leukocytes in Cibaduyut is almost similar to study result on male officers in gas station in Thailand with average number of leukocytes by 7.86×10^3/μL (Tunsaringkarn *et al*., 2013). The average number of leukocytes obtained on male workers group in Cibaduyut is still categorized as normal. Indonesian people include in Asian people group who generally have number of leukocytes more or less similar to Caucasian race which is by $3.7\text{-}9.5\times10^3$/μL for healthy adult male workers (Bain, 2006). Some other studies on workers measuring the number of leukocytes showing smaller result compare to study in Cibaduyut, one of the studies is conducted on workers in decorating section of ceramic industry in Egypt which shows that the average number of male workers leukocytes exposed to benzene is 6.14×10^3/μL and 6.54×10^3/μL on female workers (Ibrahim *et al*., 2014). Study conducted in France shows that the average number of leukocytes is 5.04×10^3/μL and 5.90×10^3/μL (Avogbe *et al*., 2011).

S-PMA level in urinary is significantly related to the number of leukocytes. The result of the study is in line with other studies which correlate urinary S-PMA level with hematology effect indicates that S-PMA in urinary is related to some blood parameters which are leukocytes, erythrocytes and neutrophils (Lan *et al*., 2004). Other studies comparing the level of S-PMA in urinary with the number of leukocytes on workers are limited because most studies correlate benzene concentration in air with the number of leukocytes (Lan *et al*., 2004; Qu *et al*., 2002; Ibrahim *et al*., 2014; Avogbe *et al*., 2011; Schnatter *et al*., 2010).

In this study, there is no significant relation between age and the number of leukocytes variables found. This study is in line with study in Japan (Nakanishi *et al*., 2003). The effect of leukocytes increase is clearly seen in various range age due to the different of smoking intensity and the number of cigarettes consumed every day (Fernandez *et al*., 2012). Variable of working time with the number of leukocytes does not show significant correlation as well. This caused by benzene concentration in indoor air with low concentration is still below quality standard and there are other factors which can affect the

number of leukocytes of every worker which is different life style like smoking habit, alcoholic intake, physical activity (Bain, 2006). Study in China shows that benzene exposure in air in concentration of <1 ppm in 16 months cause the decrease number of leukocytes by 15-18% compares to unexposed workers (Qu *et al*., 2002). This is caused by benzene exposure with concentration around >0.05 ppm and has exceeded from quality standard. In this study, there is no significant difference of average number of worker's leukocytes with working hours >8 h and ≤8 h per day found. The result of the study is in line with the study result in Japan (Nakanishi *et al*., 2003). Negative association of the number of leukocytes with long working hours may not be mediated through subclinical inflammation reaction; this is related to intensive physical activity (Nakanishi *et al*., 2003).

Some literature studies show that smoking can increase the number of leukocytes. However, the result of the study is not in accordance with theory available because there is no significant difference between the average number of leukocytes and smoker's status in this study. This is caused by most workers are light smokers. The number of leukocytes increases along with smoking intensity increase and the number of cigarettes consumed (Islam *et al*., 2007). The increase number of leukocytes on peripheral blood is in line with the more smoke exposure due to inflammation reaction inside body. This is caused by the existence of Particulate Matter of 2.5 ($PM_{2.5}$), nicotine or tar, carbon monoxide, formaldehyde and other substances. When someone experiences inflammation, then body will produce more leukocytes to fight it (Lavi *et al*., 2007). Former smokers include in light smoker category in which they have stopped smoking for one year. The former smokers who have stopped smoking for one-year experience significant decrease on some exposure biomarker including leukocytes (Roethig *et al*., 2008).

Based on the study result, there is no difference on the average number of leukocytes between workers consuming alcohol and those who are not. The study result is in line with study in Japan (Nakanishi *et al*., 2003). Alcoholic drinks pattern is measured with interview and questioner and not constitute a routine consumption but only occasional. Alcoholic drinks contain ethanol which can increase benzene metabolism and toxicity process. The interaction between ethanol and benzene also increase higher hematotoxicity effect on workers exposed to benzene who consume alcohol, compare to workers exposed to benzene but not consuming alcohol (Wilbur *et al*., 2008). Based on infection history, disease which used to be experienced in one last month is typhoid which is by 4.7% of all workers studied. Typhoid is a disease caused by *Salmonella typhii* bacteria which generally can cause the decrease of number of sufferer's leukocytes (Hoffbrand *et al*., 2005) . However, the effect of bacterial infection may have gone because during the study, workers state to have recovered from illness and look healthy as well as have worked actively.

Most workers admit to routinely exercise once a week. The types of exercise which is mostly performed by workers who routinely exercise are jogging, futsal and badminton. However, in this study there is no difference between workers who have routine exercising and those who does not have found. This is caused by the number of leukocytes which increases on a person is caused by intensive physical activity. Therefore, the result of leukocytes measurement is higher during the afternoon than in the morning (Bain, 2006). Leukocytes produced from exercising activity but are reused for recovery time due to inflammation in the body. Inflammation can be caused by workers have smoking habit. This is in line with other studies which find that someone who only walks for six minute with medium intensity experiences a significant increase on the number of leukocytes. However, due to inflamed cellular circulation increase and recovery, then the number of leukocytes will decrease significantly (Helvoort *et al*., 2007). In the study, there is no average difference of gluing worker's leukocytes with non-gluing worker leukocytes found. This is caused by most industries do not have separated room, so, most workers both in gluing and non-gluing section are positioned in the same room. In the modeling of the correlation between S-PMA level in urinary and the number of leukocytes of workers produces regression equation. After assumption test of regression equation is performed, it does not meet linearity and normality test. From the significant result on S-PMA in the research, it is recommended to explore research in in benzene exposure with biomarker of effect like reactive oxygen species, lipid peroxidation, DNA dammage and so on since, there is still few data with local characteristic of Indonesian people.

CONCLUSION

Based on the result found in this study, it can be concluded that the higher benzene concentration in indoor air, the more workers who have higher urinary S-PMA level. The the number of leukocyte of workers is still normal, however, there is association tendency that every S-PMA level increase, it can decrease the number of leukocytes after it is controlled by type of job, working hours per day and exercising habit variables.

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