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Detoxification of Benzoic Acid in Workers Exposed to Toluene Using Food Rich in Glycine

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ABSTRACT

One of the toxins found in shoe industry affecting the health of the workers is toluene. Toluene contains *safat*, a high toxicity with liver and kidneys as the main targets. This research aims to determine the effects of consuming food rich in glycine on hippuric acid levels in the urine of shoe industry workers exposed to high levels of toluene. This research is an experimental study using certain treatments for the research subjects consisting of workers exposed to toluene at some shoe home-industries. Before working, the workers were examined for hippuric acid level in their urine. The workers were then given food rich in glycine to eat. After three days of consuming food containing glycine, the workers were examined again for hippuric acid level in their urine. The findings of the study showed that the level of toluene in the shoe home-industry A was 511.8 mg/m$_3$ which was three times greater than the standard reference of the normal toluene level recommended by Permenakertrans No.13 of 2011 (168 mg/m$_3$). The results also showed that the mean concentration of hippuric acid in their urine before consuming food rich in glycine was 0.4855 g/L while the mean concentration of hippuric acid in their urine after the intake of food containing glycine was 0.649 g/L. It means that there was an increase of 33.8% in hippuric acid levels secreted in their urine. In conclusion, glycine is effective to detoxify benzoic acid from the body of workers exposed to toluene.

Keywords: *toluene, workers, benzoic acid, glycine, hippuric acid*

INTRODUCTION

Raw materials used in the production process of shoes, such as adhesives and paints, contain organic solevents. The solevents are mainly composed of benzene, xylene, ethyl benzene, toluene, and n-hexane. So that the workers can be exposed to those chemical compounds through inhalation and skin absorption. Oral intake even has also been reported in some cases.

Shoe industries use raw materials, such as toluene. This chemical material when inhaled into the body will undergo Phase I and Phase II biotransformation. Studies on human biotransformation and elimination systems have continually been developed[12]. Various clinical and in vivo studies have also been conducted to evaluate the effects of food and its components on detoxification, including phase I of cytochrome P450 enzyme and phase
II of conjugation enzymes. In Phase I biotransformation, toluene will perfectly generate benzoic acid, and it will react with glycine, part of the protein in Phase II biotransformation, to generate hippuric acid excreted through urine. This first phase of the biotransformation of toluene into benzoic acid is highly dependent on the role of cytochrome P450. Next, benzoic acid will react with glycine to generate hippuric acid, and in urine this can be used as an indicator of toluene detoxification.

Based on the results of a research conducted by the USDA[16] is used for data comparison and analisys.

MATERIAL AND METHOD

This research is an experimental study using certain treatments for the research subjects consisting of workers exposed to toluene at some shoe home-industries. Before working, the workers were examined for hippuric acid level in their urine. The workers were then given food rich in glycine to eat. Food containing glycine given to the workers includes tomatoes, beans, spinach, asparagus, garlic, tuna, and milk. Those kinds

of food in the form of vegetables, fish, and milk with the same weight were given to each worker. After three days of consuming food containing glycine, the workers were examined again for hippuric acid level in their urine. Hippuric acid level before and after the meals contained in their urine were then compared to determine the effects of food containing glycine on the level of hippuric acid excreted in their urine.

The number of the research subjects in this research was 20 workers from Tambak Oso Wilangun area. The independent variables in this research were toluene vapor exposure with a unit of mg/m_3, while the dependent variables were hippuric acid levels in urine with a unit of mg/L.

To examine the differences in the levels of toluene and hippuric acid between the treated group and the untreated group, unpaired T test was performed.

FINDINGS

a. General Description of the Research Location

Two types of glue used in the gluing process for shoe home industry: yellow glue (Super SM brand) as well as white glue and LK glue (PU-Weber, DS-Bond DNS 818). Yellow glue is used to join opening parts, and white glue is generally used to patch sole due to its stronger adhesive power. In weekdays, they can spend 30-40 kg of yellow glue and 30 kg of white glue. Glue is poured it into small containers, or by directly using the container of the glue weighed 3 kg.

The workers usually glue using their fingers directly without gloves and masks. The air condition is very hot with terrible glue fume smell. Most workers even work shirtless, smoke, eat and also rest or sleep in the same area.

The condition of the workers is more or less the same as the other shoe craftsmen in general. The working environment is poorly ordered, all section were all located in the same room together with the storage place of other raw materials. In addition, the ventilation system is inadequate and lack of exhaust fans or even without any fans.

b. Concentrations of Toluene in the Workplaces

Table 1 shows the concentrations of toluene in the shoe home-industries. The toluene concentration measurement in a unit of ppm and in temperature of celsius degrees was performed by UPT K3 Hiperkes in East Java Province. The measurement results are shown in the table below.

Table 1. Concentrations of Toluene in the Workplaces

Location	Time (Wib)	Toluena concentrations (ppm)	Dry Temperature	Temperature in o Kelvin	$_oK$ x R	#Ppm x BM	Toluena levels (mg/m_3	Note:
A	12.22	138.882	31.7	304.7	24.99	12789.5	511.8	>
B	12.35	4.246	32.8	305.8	25.075	391.9	15.6	<
C	12.50	10.763	31.9	304.9	25.042	991.6	39.6	<
D	12.59	4.413	32.4	305.4	25.042	406.6	16.23	<
E	13.07	11.264	32.6	305.6	25.059	1037.8	41.41	<
F	13.30	0.968	30.9	309.9	25.387	89.2	3.51	<
G	13.33	0.675	30.9	303.9	24.920	62.3	2.50	<
H	13.40	0.212	31.6	304.6	24.980	19.5	0.78	<
I	13.50	0.878	31.7	304.7	24.990	80.9	3.23	<

Note: NAB toluene 168 mg/m_3. (<: Less than NAB,>: greater than NAB)

Based on Permenakertrans No.13 of 2011, the standard reference of the normal toluene concentration in working environment is 168 mg/m3. As shown in Table 1 above, the highest level of toluene in location A was 511.8 mg/m3, while the smallest concentration of 0.78 mg/m3 in Location H, and the average concentration was 70.52 mg/m3.

c. Glycine Weights in Food

Types of food and meal time as indicated in the table below.

Table 2. Glycine Weights at Each Meal Time

No	**Days (Meal Time)**	**Types of Food (g)**						**Glycine Weight (g)**
1	Saturday (Breakfast)	Spinach 80 gr	Glycine weight 80/100 x 0.645= 0.516	**Pepes** tuna 100 gr	Glycine weight 1.436	-		1.952
2	Saturday (Lunch)	Stir-fried green beans 70 gr	70/172 X0,6 0.244	Leeks 33 gr	33/112 X3,47 1.022	Fried tempeh 20 gr	20/100 X1,38 0.276	2.509
3	Saturday (Dinner)	Leeks 33 g	1.022	Stir-fried yard long beans 70 gr	70/100 x1,73 1.211	Fried tempeh 20 gr	0.276	2.509
4	Sunday (Breakfast)	Yard long beans 70 gr	1.211	Leeks 25 gr	1.022	Fried tempeh 20 gr	0.276	2.509
5	Sunday (Lunch)	Tuna 100 gr	1.436	Stir-fried yard long beans 70 gr	1.211	Fried tempeh 20 gr	0.276	2.923
6	Sunday (Dinner)	Seaweed 70 gr	70/100 X 3.009= 2.11	Paprika (33 g)+ tempeh (20 g)	33/100 X0,66=0.22 + 0.276 = 0.496	Tuna 100 gr	1.436	4.43
7	Monday (Breakfast)	Yard long beans with peanut sauce 70 gr	1.211	Fried tempeh 20 gr	0.276	-		1.487
8	Monday (Lunch)	Tuna 100 gr 1.436		Green Beans 70 gr	0.244	Fried tempeh 20 gr	0.276	1.956

Based on the table above, the greatest weight of glycine consumed by the workers was on day six which was 4.43 grams. Meanwhile, the smallest was on day 7 which was 1.487 g, and the mean weight of glycine per one meal was 2,534 g.

d. The Measurement Results of Hippuric Acid Levels

The results of the measurement of hippuric acid levels before and after the administration of food containing glycine were as follow:

Table 3. Hippuric Acid Levels before and after the Administration of Food Containing Glycine

No. Sample	Hippuric Acid Levels in the urine (g/L) on June 12, 2015	Hippuric Acid Levels in the urine (g/L) on June 15, 2015
1	0.56	1.12
2	0.51	-
3	0.58	0.35
4	0.79	0.35
5	0.35	0.19
6	0.61	0.59
7	0.53	1.14
8	0.41	0.98
9	0.48	0.52
10	0.40	0.98
11	0.68	0.31
12	0.19	0.70
13	0.49	-
14	0.08	0.65
15	0.27	0.44
16	0.05	1.63
17	0.78	1.06
18	0.11	0.75
19	0.37	0.75
20	1.47	0.68
Total	9.71	11.69
Mean	0.4855	0.649

Table 3 above shows that the mean level of hippuric acid prior to the administration of food rich in glycine was 0.4855 mh/L, while the mean level of hippuric acid after the administration was 0.649 mg/L. It indicates that there was an increase in the excretion of hippuric acid after the administration of food rich in glycine. This means, it can accelerate the increase in the excretion of hippuric acid, about (0.164 g/l/0.4855) x 100% = 33.8%.

e. The Correlation between Toluene Concentrations in the Air and Benzoic Acid Concentrations in the Blood

To decrease the concentrations of toluene in home-industries, it is necessary to repair the ventilation by using natural ventilation or artificial ventilation. Natural ventilation is made by using a window with an area of at least 1/5 - 1/6 of floor space. Artificial ventilation is made by using a scrubber designed specifically for toluene vapor.

The regression equation used for the correlation between the concentrations of toluene in the air and the concentrations of benzoic acid in the blood is as follows:

$Y = 0.599x$ mg / L / h + 15.23, with $r = 0.12_3$

By using the regression equation above, it can be estimated the concentration of benzoic acid in the blood of the workers at the toluene concentration of 511.8 mg/ $m_3$was as follows:

Y = 0.59 9 (511.8) + 15.23 = 321.8 mg/L/h = 2574.4 mg/L (for 8 hours)

With the same equation for toluene concentration 0.78 mg/m_3, Y = 15.697 mg/L = 376.728 mg/L (for 8 hours), and for concentration 70.52 mg/m_3, Y = 57.47 mg/L = 459.76 mg/L (for 8 hours)

f. Concentrations of Hippuric Acid in the Urine

As shown on Table 2 above, the largest consumed glycine was 4.43 grams on day 6 while the smallest one was 1.487 g on day 7. The mean weight of glycine per one meal was 2.534 g.

The mean blood volume in a person weighed 70 kg is 5 liters. If all glycine was absorbed with an average of 2.534 grams per one meal or 7.602 grams/day, the mean level of glycine in the blood would be 7.602 g/5 liters of blood or 1.5204 g/L.

The concentration of hippuric acid in the urine before intaking was 0.4855 g/L and it became 0.649 g/L after intaking. Therefore, there was an increase in the urinary excretion of 33.8%.

Based on the guidelines[1], biological exposure index of hippuric acid in urine is 2.5 g/g creatinine; whereas, toluene level in blood is 1.0 mg/L. Meanwhile, the concentration of hippuric acid in adults is 0.44 ± 0.20 g/L which is equivalent to 0.7 g/g creatinine[14]. Unlike the previous researcher, that the exposure to 100 ppm of toluene at the end of the work shift can trigger the concentration of hippuric acid into 0.4 g/L which is equivalent to 5 g/g creatinine[11]. On the other hand, the maximal concentration of hippuric acid is 2.5 g/g creatinine with a normal range of 1.5 g/g creatinine[10].

Based on those references, the concentration of hippuric acid in urine is 0.649 g/L which is equivalent to 8.1125 g/g creatinine obtained from (0.649 g/L: 0.4 g/L) x 5 g/g. The normal concentration of hippuric acid is 2.5 g/g creatinine[1]; whereas, it is 1.5 g/g creatinine[10]. The concentration of hippuric acid in the urine of the workers in this research is considered to be abnormal.

If the standard reference by ACGIH is used, it should be lowered again to 2.5 g/g creatinine or (8.1125 to 2.5) or 5.6125 g/g creatinine or 69.1%.

The molecular weight of hippuric acid is 179.17 g/mol while the molecular weight of glycine is 75.0699 g/mol. In addition, the molecular weight of benzoic acid is 122 g/mol. The mean molecular weight of hippuric acid excreted is 0.0036 mol/L derived from 0.649 g/L
: 179.17 g/mol. The mean molecular weight of benzoic acid is 459.76 mg/L or 0.45976 g/L which is equal to 0.0038 mol derived from 0.45976 g/L: 122 g/mol.

It can be said that for benzoic acid at a mean concentration of 0.0038 mol /L, the concentration of glycine that must be taken is 0.0038 mol /L in 8 hours in order to excrete entire benzoic acid into hippuric acid. As a result, for glycine with a molecular weight of 75.0699 g/mol, the weight of glycine needed is 0.285 g within 8 working hours, obtained from 0.0038 mol /L x 75.0699 g/mol. Meanwhile, the mean weight of glycine given to the research subjects in each meal was 2,534 g at breakfast, lunch, and dinner. If the normal weight of glycine in one meal is 2.534 grams within 8 working hours, that there is an excess of about 2.249, obtained from 2.534 g - 0.285 grams, in the food given to the research subjects to lower the levels of toluene and benzoic acid from their body.

For those reasons, for those workers in the work environment containing the highest toluene concentration of 511.8 mg/m_3 with the benzoic acid concentration of 2574.4 mg/L or 2.5744 g/L, equivalent to 0.021 mol/L, as much as 0.021 mol/L glycine which is equal to 15.765 gram glycine derived from 0.21 mol/L x 75.0699 g/mol is required to lower the benzoic acid concentration. The portion of food containing glycine needs to be increased to 15.765 g / 2.534 g or 6.22 times greater than the mean portion of glycine given during the research.

CONCLUSION

Based on the results of this research, it can be concluded that:

a. The mean concentration of toluene in this research was 70.52 mg/m_3 which was still below the standard reference of the normal toluene concentration recommended by Permenakertrans No.13 of 2011 (168 mg/m_3). The level of toluene in the shoe home-industry A was 511.8 mg/ m_3. This high concentration of toluene in the shoe home-industries was due to poor ventilation.

b. The mean concentration of hippuric acid in the urine before intaking food rich in glycine was 0.4855 g/L while the mean concentration of hippuric acid in the urine after intaking food containing glycine was 0.649 g/L. It means that there was an increase in the urinary excretion of about 33.8%.

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Ethical Clearance: The study was approved by the institutional Ethical Board of the Public Health, Airlangga University.

All subjects were fully informed about the procedures and objectives of this study and each subject prior to the study signed an informed consent form.

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