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# FACTORS INFLUENCING URINARY METABOLITES AMONG FACTORY EMPLOYEES EXPOSED TO TOLUENE AND XYLENE IN THAILAND.

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## ABSTRACT

The aim of this study was to determine factors influencing urinary metabolites among factory employees exposed to toluene and xylene. Data were collected from 118 employees exposed to mixed solvents in 4 factories, and 100 non-exposed employees in a frozen-food factory. Demographic information was obtained by interview. Exposure was determined by personal air samplers and urinary metabolite levels. The average age of the study group was 32.8 (7.3) years. The mean concentration of toluene was 7.50 (9.92) ppm, and xylene was 2.17 (2.30) ppm, respectively. The t-test found significant differences between the exposed and control groups for concentrations of toluene and xylene ($p< 0.001$). Significant positive associations were found between concentration of toluene and urinary hippuric acid among exposed male and female employees (95% CI = 8.24, 23.21 and 95% CI = 5.61, 80.14). Xylene was associated with urinary concentration of methyl hippuric acid among the whole subject group and the exposed male employees (95% CI = 11.13, 22.29, and 95% CI = -7.37, 133.30), respectively. The results suggested that urinary hippuric acid remained a useful biomarker of toluene exposure among the employees exposed to mixed solvents, and methyl hippuric acid to xylene exposure.

**Keywords:** Toluene and xylene, hippuric acid, methyl hippuric acid and urinary metabolites



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## INTRODUCTION

Toluene and xylene are organic solvents widely used in factories in Thailand as raw materials, additives, and cleaning agents (Todd et al., 2008). Under working condition, employees can be exposed to toluene and xylene from workplace ambient air. In Occupational health, toluene and xylene exposure level have largely been evaluated by measuring workplace ambient air concentrations (Domański and Makles, 2012). Because toluene and xylene are metabolized as hippuric acid and methyl hippuric acid, several studies have been conducted to determine metabolic interaction between toluene and xylene exposed in workplace ambient air and hippuric acid and methyl hippuric acid in urine (Hellquist et al., 1983; Baelum et al., 1985; Boey et al., 1997). Biomarkers are very useful tools when the metabolic fate of the toluene and xylene of a resultant disease is understood. Then, evaluating the association between toluene and xylene concentrations in the workplace ambient air and metabolites (hippuric acid and methyl hippuric acid) in the urine of employees is very important (Wilczok and Bieniek, 1978; Chen et al., 1994).

Several studies have been investigated concerning the relationship between exposure to toluene and xylene and in vivo, and found a positive relationship between exposure to these solvents and metabolite levels in urine, and these were used as indicators of toluene and xylene exposure (Pagnotto and Leberman, 1967; Chen et al., 1994; Gericke et al., 2001; Hui et al., 2009; Bogovski et al., 2007; Hopf et al., 2012; Wiwanithit et al., 2002).

Several factors are known to affect the concentration of metabolic products from toluene and xylene in urine, such as alcohol consumption (Huang et al., 1994; Maestri et al., 1997). Wiwanitkit et al. (2002) reported that smoking did not influence urinary hippuric acid levels in the study group. In Thailand, few studies have been conducted on factory employees' occupational exposure to organic solvents (Wiwanitkit et al. 2002; Srisupab, 2004; Fongsupa, 2009).

To date, there is no study conducted on co-factors in association with hippuric and methyl hippuric in urine. The objectives of this study were to determine factors influencing levels of urinary hippuric acid and methyl hippuric acid among employees exposed to mixed solvents in Thailand. The results of this study could be used as part of a health-screening process for employees exposed to toluene and xylene.

## MATERIALS AND METHODS

***Study design***

This cross-sectional study assessed urinary metabolites, hippuric acid and methyl hippuric acid in the exposed subjects who had been exposed to mixed organic solvents, mainly mixed with Toluene and Xylene at paint manufacturing plants, container-cleaning plant, and auto-part manufacturing, as well as the control group in a food manufacturing factory. Data were collected in April-June 2011.

***Study sites and samples***

The study sites and samples were classified into three groups, i.e., the exposed group (group 1) comprised subjects who had been exposed to mixed organic solvents at paint manufacturing plants # 2 (58 subjects) and # 3 (31 subjects). Group 2 comprised subjects from container-cleaning plant # 4 (14 subjects) and auto-part manufacturing plant # 5 (15 subjects). The control group (group 3) comprised 100 randomly selected employees unexposed to organic solvents, in a food-manufacturing factory.

Inclusion criteria

The inclusion criteria for the exposed group of participants were as follows: the first group of participants was 20-60 years of age, the second group of participants was those who worked in a process exposed to organic solvents, and the third group of participants was the employees who volunteered to participate in this research with informed consent. The study protocol was approved by the Research Ethics Committee of Burapha University.

***Research tools and instrumentation***

The research tools and instrumentation used in this study were questionnaires and passive badge samplers, gas chromatography, and high performance liquid chromatography (HPLC). The constructed questionnaires consisted of demographic and job-related questions, i.e., age, sex, alcohol consumption, history of work exposed to organic solvents, and self-protection behaviors, especially wearing a cartridge respirator. A passive badge sampler (3M 3500 Organic Vapor Monitor) was used for air sampling. The gas chromatography, FID Column Aqua-Wax Polyethylene Glycol (size 30 m x .25 mm x 0.25 μm), was used to analyze toluene and xylene concentrations from the passive badges. The calibration and quality control were carried out by following the NIOSH method 1501(Fourth edition (NIOSH, 1994).

High performance liquid chromatography (HPLC) was used to analyze the urinary concentrations of hippuric acid and methyl hippuric acid. A spectrophotometer was used to analyze creatinine (Star Dust MC 15). The calibration and quality control were carried out using the NIOSH method 8301 (NIOSH, 1994).

***Data and sample collection***

A questionnaire interview was administered to each subject. Questions included demographic and job-related data--age, sex, alcohol consumption, history of work exposure to organic solvents, self-protection behaviors, especially wearing a cartridge respirator. Toluene and xylene concentrations in the workplace ambient air were assessed by personal air sampling, in which a passive diffusion device was attached to the collar of the employee's shirt during an 8-hour work period. Samples were immediately transferred by ice box and kept in a freezer at -20 $^{o}$C in the laboratory of the Faculty of Public Health, Burapha University. The concentrations of toluene and xylene were analyzed by gas chromatography at the Occupational and Environmental Department, Ministry of Public Health, Bangkok. Analysis of samples was conducted using NIOSH method 1501, fourth edition. The measurement precision was 0.11 for toluene and 0.10 for xylene.

In case of urine samples, at least 10 ml per person of urine were collected after 8 hours at the work station. The sample was immediately placed in an ice box for transfer to a -20 $^{o}$C freezer in the laboratory at the Faculty of Public Health, Burapha University. High performance liquid chromatography (HPLC) and spectrophotometry (Star Dust MC 15) were used to analyze concentrations of urinary hippuric and methyl hippuric acid, and creatinine, at the Occupational and Environmental Department, Ministry of Public Health, Bangkok. Analysis of samples was conducted using NIOSH method 8301, fourth edition.

***Data analysis***

Statistical software was used to analyze the demographic and work-related data: age, sex, alcohol consumption, respirator use, number of years in trade, and job-related exposure. Concentrations of toluene, xylene, level of hippuric acid and methyl hippuric acid in urine were analyzed using descriptive statistics (frequency, percentage, mean, SD, median, (maximum and minimum), respectively.

The t-test was used to analyze differences between the exposed and non-exposed groups for urinary concentrations of toluene, xylene, hippuric acid and methyl hippuric acid concentrations. Multiple linear regression was used to analyze multifactorial demographic and work-related data: age, sex, alcohol consumption, respirator use, number of years in trade, and job-related exposure; urinary concentrations of toluene, xylene (which influence hippuric acid and methyl hippuric acid concentrations in urine) (CI=95%), respectively.

## RESULTS

**Demographic information**

The total sample population was 118 employees, of whom most of them (106 persons, 89.8%) were males; the mean age was 32.8 (7.3) years. Most of them (88 persons, 74.6%) consumed alcohol beverage; of these, 17 (19.3%) drank one time per week, while 30 (34.1%) drank 2-3 times per week.

From the interviews about current job-related exposure, over half of the employees (61 persons, 51.7%) had high-level exposure to organic solvents and 16 (13.6%) had moderate-level exposure. The mean number of years in the trade was 6.4 (5.9) years. Almost all of them used personal protective equipment (PPE) when exposed to organic solvents (113 persons, 95.8%). Most of them wore cartridge respirators while working (94 persons, 83.2%).

**Assessment of toluene and xylene concentrations in the work environment, and hippuric acid and methyl hippuric acid in urine**

One hundred and eighteen (118) air samples were collected. Analysis showed that 64 (54.2%) had concentrations of toluene in the range of 1-10 ppm, and 24 (20.3%) had < 1 ppm. Mean (SD) and median concentrations (min-max) of toluene were 7.50 (9.92) ppm and 3.32 (0.17-48.67) ppm, respectively. Similarly, 66 samples (55.9%) showed concentrations of xylene at 1-10 ppm, and 51 samples (43.2%) showed concentrations of xylene at < 1 ppm. Mean and median of concentrations of xylene were 2.17 (2.30) ppm and 1.43 (0.09-13.08) ppm, respectively. The results showed that the mean (SD) and median concentrations (min-max) of hippuric acid were 448.68 (375.51) mg/g creatinine and 364.30 (5.46-1911.92) mg/g creatinine respectively; and for methyl hippuric acid, they were 69.99 (73.53) mg/g creatinine and 44.48 (0.30-331.70) mg/g creatinine, repectively (Table 1).

**Table 1.** Concentration of hippuric acid and methyl hippuric acid in urine between exposed group and non-exposed group

| Concentration | Exposed (n=118) | Non-exposed(n=98) |
|---|---|---|
| Hippuric acid (mg/g creatinine) | | |
| Mean(SD) | 448.68 (375.51) | 202.18 (188.70) |
| Median (min-max) | 364.30 (5.46-1911.92) | 166.45 (1.00-1066.07) |
| Not detected | 56 (47.5%) | 34 (34.7%) |
| Methyl hippuric acid (mg/g creatinine) | | |
| Mean(SD) | 69.99 (73.53) | 33.72 (32.44) |
| Median (min-max) | 44.48 (0.30-331.70) | 25.43 (4.60-168.49) |
| Not detected | 38 (32.20%) | 71 (72.4%) |



The evaluation of toluene and xylene concentrations in the workplace ambient air, classified by gender, was based on 118 samples from four factories. The mean toluene level among the exposed male and female employees were 7.50 ppm (95% CI; 5.57-9.42), 7.52 ppm (95% CI; 1.74-13.31), respectively. The mean xylene concentrations in the exposed male and female employees were 2.35 ppm (95% CI; 1.89-2.80) and 0.61 ppm (95% CI; 0.22-1.00), respectively. There was no difference between toluene and xylene concentrations in the workplace ambient air between male and female employees. However, the differences between toluene and xylene concentrations in the workplace ambient air for the exposed and non-exposed groups were significant (p-value<0.001) (Table 2).

Assessment of the urinary concentrations of hippuric acid in the exposed group was based on 118 samples from four industries. The mean hippuric acid level among the exposed male and female employees were 448.04 (95% CI; 348.90-547.18) mg/g creatinine and 456.06 (95% CI; 93.29 -1005.41) mg/g creatinine, respectively. The difference between the mean hippuric acid concentrations for the exposed and non-exposed groups was significant (p-value<0.001) (Table 1).

Urine concentration assessment of methyl hippuric acid for the exposed group was based on 118 samples (4 industries). The mean methyl hippuric acid concentration among the exposed male and female employees were 74.77 mg/g creatinine (95% CI; 57.43-92.11) and 33.47 mg/g creatinine (95% CI; 9.41-76.36), respectively. There was no difference between mean concentrations of methyl hippuric acid for the exposed and non-exposed groups (Table2).

**Table 2.** Concentration of exposure between the exposed and non-exposed group

| Type of chemical/ urine metabolites | Exposed (n=118) | | Non-exposed (n=47) | | Total (n=165) | |
|---|---|---|---|---|---|---|
| | Male x□ (95% CI) | Female x□ (95% CI) | Male x□ (95% CI) | Female x□ (95% CI) | Exposed x□ (95% CI) | Non-exposed x□ (95% CI) |
| Toluene (ppm) | 7.50 (5.57, 4.42) | 7.52 (1.74, 13.31) | 0.26 (0.18,0.34) | 0.56 (0.27,0.86) | 7.50 (5.69,9.31) | 0.49 (0.26,0.71) |
| Xylene (ppm) | 2.35 (1.89,2.80) | 0.61 (0.22, 1.00) | 0.31 (0.30,0.33) | 0.32 (0.31,0.33) | 2.17 (1.75,2.59) | 0.32 (0.310,0.33) |
| Hippuric acid (mg/g creatinine) | 448.04 (348.90, 547.18) | 456.06 (93.29,1005.41) | 242.64 (135.20,350.07) | 178.47 (130.88,226.06) | 448.68 (353.32,544.04) | 200.19 (153.24,247.131) |
| Methyl hippuric acid (mg/g creatinine) | 74.77 (57.43, 92.11) | 33.47 (9.41, 76.36) | 46.18 (8.64,83.72) | 27.49 (18.00,36.97) | 70.74 (54.55,86.93) | 33.72 (20.88,46.55) |

**Factors affecting urinary concentrations of hippuric and methyl hippuric acid**

*Hippuric acid:* multiple linear regression analysis for sex, age, alcohol consumption, respirator use, number of years in trade, job-related exposure, concentration of toluene (ppm) and hippuric acid showed that toluene concentration in the workplace ambient air was associated with urinary concentration of hippuric acid among exposed male and female employees. When the mean toluene concentration in the workplace ambient air was increased by 1 ppm, the urinary concentration of hippuric acid in male and female was increased by 15.726 mg/g creatinine (95% CI = 8.24, 23.21) and 42.872 mg/g creatinine (95% CI = 5.61-80.14), repectively (Table3).

*Methyl hippuric acid:* multiple linear regression analysis of sex, age, alcohol consumption, respirator use, number of years in the trade, job-related exposure, and concentrations of xylene (ppm) and methyl hippuric acid showed that xylene concentration in the workplace ambient air was associated with urinary concentration of methyl hippuric acid in exposed male employees. When mean xylene concentration in the workplace ambient air was increased by 1 ppm, the urinary concentration of methyl hippuric acid was increased by 16.711 mg/g (95% CI = 11.13, 22.29). However, xylene concentration had no influence on urinary methyl hippuric acid level among exposed female employees (Table 4).

## DISCUSSION

The mean concentration of toluene in this study was 7.5 (9.92) ppm which is consistent with the study of Srisupab, at 7.84 ppm (Srisupab, 2004). However; it was higher than Hopf et al. (2012) where the geometric mean toluene was 0.05 ppm. On the other hand, it was less than Chen et al. (1994), at 203 ppm.

The 8-hour mean concentration of xylene in the workplace ambient air was 2.17(2.30) ppm. This level was below the ACGIH recommenced value (ACGIH, 2010) , but it was greater than the study of Hopf et al. (2012) where the geometric mean was 0.06 ppm. However, the mean concentration in the present study was lower than the studies by Chen Z et al. (1994) at 103.0, Ukai et al. (2007) at 1.0, and Mao et al. (2007) at 72.63 (13.37) ppm.

**Table 3.** Factors of sex, age, drinking history, respirator used, number of year in trade, job-related exposure, and concentration of toluene (ppm) associated with hippuric acid

| Factors | All #1 β | (n=90) (95% CI) | Male #2 β | (n=76) (95% CI) | Female #3 β | (n=16) (95% CI) |
|---|---|---|---|---|---|---|
| Constant | 94.381 | (-291.13, 479.89) | 73.385 | (-545.11, 691.87) | 266.069 | (-331.07, 863.20) |
| Exposed group | 5.288 | (-191.01, 201.59) | 7.677 | (-244.13, 259.48) | -124.411 | (-487.51, 238.68) |
| Gender (male) | 20.468 | (-168.87, 209.80) | | | | |
| Age (yrs) | 0.997 | (-8.13, 10.13) | 2.609 | (-10.78, 16.00) | -4.214 | (-19.07, 10.64) |
| Drinking alcohol (yes) | 76.904 | (-72.85, 226.65) | 63.114 | (-144.24, 270.47) | 83.822 | (-324.64, 492.29) |
| Cartridge (yes) | -14.274 | (-170.08, 141.53) | -16.191 | (-196.98, 164.60) | 6.214 | (-677.55, 689.98) |
| Trade 1-5 yrs | 24.922 | (-274.79, 324.63) | 38.409 | (-340.87, 417.69) | 14.036 | (-501.45, 529.52) |
| Trade >5 yrs | 86.731 | (-232.08, 405.54) | 84.952 | (-335.11, 505.01) | 55.339 | (-473.94, 584.62) |
| History of solvent exposure (moderate to high) | 34.267 | (-126.18, 194.71) | 27.143 | (-159.54, 213.82) | -8.959 | (-788.88, 770.96) |
| Toluene concentration (ppm) | 16.453 | (9.82, 23.08) | 15.726 | (8.24, 23.21) | 42.872 | (5.61, 80.14) |

All #1 R2 = 36.30%, p-value<0.001; Male #2 R2=31.4%, p-value=0.003; Female #3 R2=55.0%, p-value=0.061

**Table 4.** Factors of sex, age, drinking history, respirator used, number of year in trade, job-related exposure, and concentration of xylene (ppm) associated with methyl hippyric acid

| Factors | All #1 β | (n=90) (95% CI) | Male #2 β | (n=76) (95% CI) | Female #3 β | (n=16) (95% CI) |
|---|---|---|---|---|---|---|
| Constant | -35.298 | (-123.42, 52.82) | 68.049 | (-92.38, 228.47) | -101.493 | (-243.89, 40.90) |
| Exposed group | -39.598 | (-98.53, 19.33) | -144.608 | (-268.95, -20.27) | -12.180 | (-101.98, 77.62) |
| Gender (male) | 19.053 | (-23.98, 62.09) | | | | |
| Age (yrs) | 1.560 | (-0.59, 3.71) | 1.860 | (-0.81, 4.53) | 2.414 | (-0.86, 5.69) |
| Drinking alcohol (yes) | 20.918 | (-10.02, 51.86) | 36.283 | (0.73, 71.83) | -17.266 | (-71.62, 37.09) |
| Cartridge (yes) | -0.714 | (-33.41, 31.99) | -0.868 | (-34.60, 32.87) | | |
| Trade 1-5 yrs | -0.595 | (-49.87, 48.68) | -3.970 | (-58.05, 50.11) | -0.868 | (-98.62, 96.89) |
| Trade >5 yrs | 17.315 | (-34.88, 69.51) | 11.677 | (-48.32, 71.67) | 39.407 | (-53.97, 132.78) |
| History of solvent exposure (moderate to high) | 11.778 | (-15.41, 38.97) | 17.041 | (-12.88, 46.96) | -3.508 | (-83.79, 76.78) |
| Xylene concentration (ppm) | 17.230 | (11.83, 22.63) | 16.711 | (11.13, 22.29) | 62.967 | (-7.37, 133.30) |

All #1 R2 = 35.5%, p-value<0.001; Male #2 R2=37.3%, p-value<0.001; Female #3 R2=73.1%, p-value=0.162

The urinary concentration of hippuric acid was used as biological indicator to measure toluene in the workplace ambient air; this is a very useful method of screening those who work with organic solvents. In this study, it was found the hippuric acid concentrations of the exposed and non-exposed groups were significantly different (p value<0.001). This result is similar to the study of Heuser et al. (2007) who found that urinary concentrations of hippuric acid among toluene-exposed employees in a shoe factory differed from non-exposed employees (p-value<0.001).

The mean hippuric acid level of 448.68 (375.51) mg/g creatinine was lower than the ACGIH (2010) recommended value, but it was higher than the study of Shih et al. (Shih et al., 2011) where the mean was 0.34 (0.18) mg/g creatinine. In this study, it was also found that the concentration of hippuric acid differed significantly between Monday and Friday (OR= 4.13, p=0.01). The hippuric concentrations in this study were also lower than those detected in glue factories, at 1.240 mg/g creatinine (Gargouri et al., 2011).

Previous studies showed that evaluating exposure to toluene in the urine is a more sensitive indicator than the metabolite forms of hippuric acid and/or ortho-cresol (Janasik et al., 2008); however, after controlling the factors of age, sex, alcohol consumption, respirator use, number of years in the trade, and job-related exposure in this study, the result showed that toluene concentrations in the workplace ambient air was significantly correlated with urinary hippuric acid levels among male and female employees (p-value<0.001). Multiple linear regression analysis showed that concentration of toluene in the workplace ambient air affected urinary hippuric acid levels among male and female employees. When toluene in the workplace ambient air was increased by 1 ppm, urinary hippuric acid concentrations in male and female employees were increased, respectively, by 15.726 mg/g creatinine (95% CI = 8.24, 23.21), R2 = 31.4%, p value<0.003 and by 42.872 mg/g creatinine (95% CI = 5.61, 80.14), R2 = 55.0%, p value<0.061.

This evidence confirms that hippuric acid remains a valid indicator for measuring toluene exposure levels in the work environment among male and female employees. This finding concurs with several studies, e.g., the study of toluene in plastic balls by Fongsupa (2009) which showed that toluene concentration in the workplace ambient air correlated with urinary hippuric acid. Hopf et al. (2012) evaluated offshore employees exposed to low concentrations of toluene, and showed a relationship between toluene in the workplace ambient air and urinary hippuric acid level (p-value<0.001). Ukai et al. (2007) showed a similar correlation between toluene concentration in the work environment and urine level (r =0.85).In 2010, Janasil et al. found that toluene concentration in the work environment was correlated with toluene concentrations in both blood and urine (p-value<0.05) (Janasil et al., 2010). Huang et al. (1994) found a correlation between concentration of toluene during the 8 working hours and urinary hippuric acid in urine.

The current study found that respirator use had no effect on urinary concentration of hippuric acid. This disagrees with the study of Ogata et al. (1971) who found that the toluene concentration in a toluene-exposed respirator was less than those not exposed to solvents in expired air, blood, or urine. In the present study, employees tended to wear cartridge respirators as PPE only when exposed to high concentrations of organic solvents; however, these substances were always dispersed in the workplace ambient air, and so employees were still at risk of exposure.

The mean amount of urinary methyl hippuric acid found after work was 69.99 (73.53) mg/g creatinine. This value was below the ACGH standard (2010), but it did not differ between exposed and non-exposed groups. This may be due to the decomposition of food (Maestri et al., 1997). Alcohol consumption had no effect on urinary methyl hippuric acid concentration, which was contrasted with the study of Huang et al. (1994) who reported a correlation between xylene concentration in the workplace ambient air and urinary methyl hippuric acid, and found that the rate of degradation of xylene in urine was decreased in smokers and drinkers.

The current study showed that xylene concentration was an influencing variable in the prediction of urinary methyl hippuric acid concentrations in the whole subject group and the exposed male employees. For the whole group, an increase of 1 ppm xylene concentration in the workplace ambient air was reflected in urinary methyl hippuric acid increase of 17.23 mg/g creatinine (95% CI = 11.83, 22.63), R2 = 35.5; for male exposed employees, an increase of 1 ppm xylene concentration in the workplace ambient air was reflected in an increase of urinary methyl hippuric acid by 16.711 mg/g creatinine (95% CI = 11.13, 22.29), R2 = 37.3.

Methyl hippuric acid remains a suitable biomarker for employees exposed to xylene in the workplace ambient air which is consistent with the study of de Carvalho et al. (1991). However, the number of methyl hippuric acid samples among female employees was too small. When controlling factors in linear regression model, the power was decreased, then xylene concentration in the work environment and urinary methyl hippuric acid among female had no association but very likely.

The main limitation of this study was that the concentrations of studied substances in the samples were too low (below the instrument detection limit); therefore, some values were missing from data analysis. The results of this study cannot be generalized to the entire factory setting.

In summary, urinary hippuric acid and methyl hippuric acid can be useful as biomarkers of toluene and xylene exposure. It is suggested that urinary hippuric acid can still be used as a biomarker of exposure to toluene among male and female employees, and methyl hippuric acid to xylene among male employees exposed to mixed solvents. Although other factors were not associated with the urinary metabolites studied, the authors were aware of the limited durations of employees' exposure to organic solvents; wearing a respirator must be encouraged to reduce exposure. Future studies might be focused on biochemical screening for health effects on employees exposed to mixed solvents.

Potential conflicts of interest: None

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