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Abstract- Inhalant abuse is defined as an intentional inhalation of solvents or volatile substances present in materials such as glues and paints. The most commonly abused inhalant today is glue due to low price and easy access. Glue sniffing produces fast and pleasurable sensory experience to the abuser. Quantitative analysis of hippuric acid using UV-Vis at wavelength 417 nm was determined successfully. The results gave a regression coefficient of 0.994. The recovery, accuracy and coefficient variance of hippuric acid were 96.57%, 2.94% and 0.50% respectively. Reversed-phase high performance liquid chromatography using a simple method for the simultaneous determination of hippuric acid and benzoic acid was also described. The chromatography was performed on a Nova-Pak C18 (3.9 x 150 mm) column with a mobile phase of methanol: water: acetic acid (20:80:0.2) using UV detection at 254 nm. The calibration of standards was linear within concentration range of 0.125 to 6.0 mg/mL of hippuric acid and benzoic acid respectively.

Keywords: *glue, hippuric acid, color test, ultra violet-visible.*

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Qualitative and Quantitative Analysis for Determination of Glue Sniffer's Urine

Abdul Rahim Yacob [α] & Mohamad Raizul Zinalibdin [σ]

Abstract- Inhalant abuse is defined as an intentional inhalation of solvents or volatile substances present in materials such as glues and paints. The most commonly abused inhalant today is glue due to low price and easy access. Glue sniffing produces fast and pleasurable sensory experience to the abuser. Quantitative analysis of hippuric acid using UV-Vis at wavelength 417 nm was determined successfully. The results gave a regression coefficient of 0.994. The recovery, accuracy and coefficient variance of hippuric acid were 96.57%, 2.94% and 0.50% respectively. Reversed-phase high performance liquid chromatography using a simple method for the simultaneous determination of hippuric acid and benzoic acid was also described. The chromatography was performed on a Nova-Pak C18 (3.9 x 150 mm) column with a mobile phase of methanol: water: acetic acid (20:80:0.2) using UV detection at 254 nm. The calibration of standards was linear within concentration range of 0.125 to 6.0 mg/mL of hippuric acid and benzoic acid respectively. The recovery, accuracy and coefficient variance of hippuric acid were 104.54%, 0.2% and 0.2% and for benzoic acid were 98.48%, 1.25% and 0.60% respectively. A mobile G.S. Kit was developed which employed a mixture of pyridine, benzenesulphonyl chloride and distilled water use as quantitative analysis. Urine samples containing hippuric acid the metabolite of toluene were analyzed using the G.S. Kit. The results show that the mixture would change its color from yellow to red. This method was successful in screening urine samples of suspect toluene abusers or glue sniffers among secondary school children at Johor Bahru and suspect abuser from Hospital Sultanah Aminah with the collaboration of the National Anti Drug Agency.

Keywords: glue, hippuric acid, color test, ultra violet-visible.

I. Introduction

Toluene is also known as methyl benzene or phenyl methane. It is a clear and water insoluble liquid with typical smell and redolent of sweet smell of existed compound of benzene [1]. This chemical is widely used organic solvent in the printing, painting, automotive, shoemaking, adhesive material and the pharmaceutical industries [2]. Normally, toluene is found in many products including paint and contact adhesives as a solvent. Besides that, some grades of toluene contain traces of xylene and benzene [1]. Toluene has a lower boiling point, flammable chemical and easy evaporated. It also the common substance in glue and thinner sniffed by drug abusers.

In Malaysia, the abuse of organic volatile solvents has been observed since the early 1980s. The problem of solvent abuse is predominant in East Malaysia (i.e., Sabah and Sarawak) and in Johore the southern part of West Malaysia bordering Singapore as reported by Navaratnam et, al. (1988) [3]. The trend of inhalant abuse in Malaysia has remained stable during the last 5 years, in contrast to other countries in the region, especially Thailand and Singapore. The types of substances abused include paint thinner, nail polish remover, gasoline, and glue. The most commonly abused inhalant is glue. Preventive education on the various aspects of inhalant abuse and its harmful effects has been carried out as part of preventive education on drug abuse in Malaysia. In the affected states, pamphlets on inhalant use have been produced by the departments of education for use in schools. Resource guides on inhalant abuse have been produced by the Government for health and welfare professionals and teachers.

Detected inhalant abusers of school age are provided counseling by teachers and subjected to disciplinary action. Police report nonschool children to parents for supervision. Presently, no legislation in Malaysia relates specifically to the abuse of inhalants. Nonetheless, existing legislation (i.e., the Juvenile Courts Act of 1947) could be used to a limited extent to help children or adolescents younger than 16 years old who are inhalant abusers. They can be placed in an approved home by the juvenile court. The police also can charge inhalant abusers under the Minor Offense Ordinance of 1955 if they also disturb the peace in the process of abusing.

Recently in next decade this activities rise back by newspaper because of three teenagers aged 18 to 20 were found dead near a school in Cheras, Kuala Lumpur and Police found three can of glue nearby [5]. With the info from these newspapers and previous data it is feared that this problem lead to "time bomb" disasters for future generation in Malaysia.

Toluene can be absorbed into the blood flow from the lung and the gastrointestinal tract and through the skin and mucosa. Brain and liver serve as reservoir for toluene [6]. Following inhalation or oral exposure to toluene, approximately 60 – 75% of absorbed toluene is metabolite to benzoic acid [7, 8]. Please refer to figure

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1. The initial step involves side chain oxidation to benzyl alcohol by cytochrome P450 enzymes. Benzyl alcohol is then further oxidized to benzoic acid by alcohol dehydrogenase and aldehyde dehydrogenase. Benzoic acid is subsequently conjugated with glycine to form hippuric acid [7] the reaction in a figure 2. Benzoic acid may also be conjugated with glucuronic acid to form benzoyl glucuronide in the urine. Less than 1% of absorbed toluene undergoes ring hydroxylation to form o-, and p-creosol, which are excreted in the urine as glucuronide or sulphate conjugates [9, 10,11].

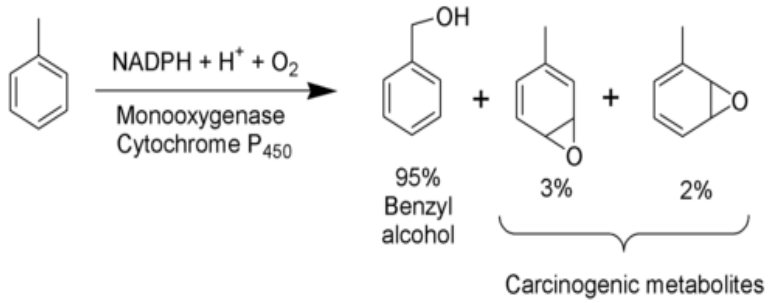

Figure 1 : First step of toluene metabolite

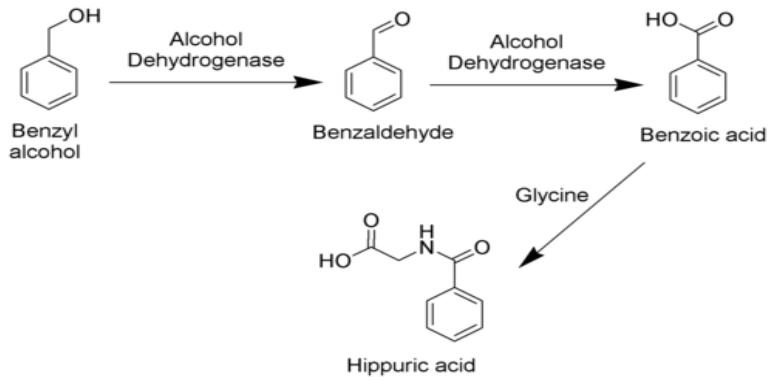

Figure 2 : Second step of toluene metabolite

Novel color reaction for hippuric acid has been developing since 1950 and keep continue in early 1980. The first color reaction developing by Gaffney et al (1954) use based upon azlactone formation resulting from the reaction of an aldehyde with hippuric acid. In this instance, hippuric acid was converted to a deep orange colored azlactone, 2-phenyl-4-(p-dimethylamino) benzal-5-oxazolone by treatment of hippuric acid with acetic anhydride and p-dimethylaminobenzaldehyde [12]. Later, in 1960 Charles J.U found out new method using pyridine and benzenesulfonyl chloride to develop deep red color in present of hippuric acid [13].

The novel color reaction will be applied in this research to determine quantitatively metabolite of toluene which is hippuric acid using Ultra Violet Visible (UV-Vis). The method of color test are using pyridine and benzenesulphonyl chloride was yellow and became reddish by addition of distilled water has been reported by Manabu Yoshida et al (2005). Actually, this novel color reaction has been developing by Charles J.U. However, this color test has been modifying by Manabu Yoshida with adding distillated water to see the color change using naked eye. This novel color reaction will be use as a screening test for glue abuser among the secondary school in Malaysia and might be help the National Anti Drug Agency and Royal Police of Malaysia to prevent the glue abuser among student and teenagers.

II. Experimental

a) Reagents

Hippuric Acid 98%, Benzenesulphonyl chloride (BSC) 98%, Toluene 99.3%, and pyridine 98% purchased from Sigma Aldrich.

b) Instrument

Ultra Violet Visible (UV-Vis) Perkin Elmer.

c) Determination of Hippuric Acid using UV-Vis.

i. *Sample Selection*

Sample urine received from screening using colour test at secondary school around Johore Bahru, Malaysia.

ii. *Sample Preparation*

a. *Urine Sample*

Urine sample of 0.1 ml will be added to 0.25 pyridine and 0.1 ml BSC with 1.0 ml distilled water

b. *Preparation of Standard Hippuric Acid*

12.5 mg, 25.0 mg, 50.0 mg, 100 mg, 150 mg and 200 mg of hippuric acid will be weighed into 100 ml

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volumetric flask. The different volumetric flask will be label as standard HA 0.125, HA 0.25, HA 0.5, HA 1.0, HA 1.5 and HA 2.0. The solution will be mark up with distilled water. All standard will be sonicated in a sonicator waterbath. All standard will be run with the UV-Vis Perkin Elmer at wavelength 243 nm. Calibration curve of hippuric acid will develop to get the significance of concentration hippuric acid with absorbance.

III. Results and Discussion

a) Result for Metabolite of Toluene

i. *Colour test for Hippuric Acid*

Table 3.1.1 : Concentration using color test method of hippuric acid at 417 nm wavelength

Concentration of Hippuric Acid	Absorbance
0.000	0.000
0.125	0.149
0.250	0.284
0.500	0.337
1.000	0.654
1.500	0.797
2.000	0.993
3.000	1.500
6.000	2.667

The UV-Vis of the mixtures were recorded 300 to 700 nm using Perkin Elmer Ultra Violet Visible (UV-Vis). A color chart for the semiquantitation of hippuric acid was prepared using hippuric acid standards containing 0.125 mg/ml to 6.00 mg/ml hippuric acid. Tables 3.1.1 show the concentration using color test method of hippuric acid at wavelength 417 nm. Based on the table, this color test method has successfully determined the presence of hippuric acid at different concentration. These results proved that this method might be applied to detect hippuric acid.

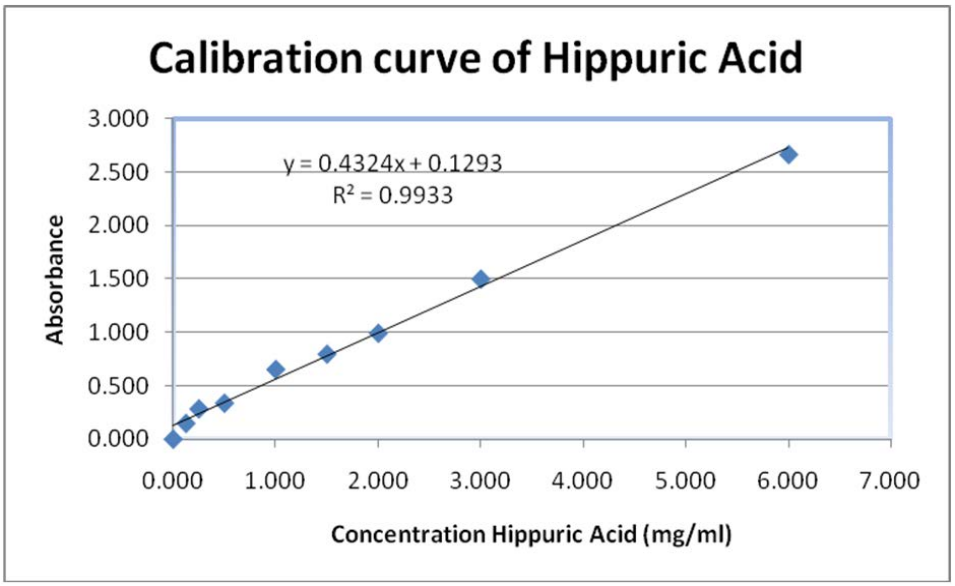

Figure 3.1.1 : Calibration curve of hippuric acid using colour test method

From the calibration curve of hippuric acid using the colour test method, the R2=0.9933 and the amount hippuric acid will be calculate for the equation y=0.4324x + 0.1293. Based on the graph the amount of hippuric acid in the urine can be calculated and shown in the Table 3.1.4.

ii. *Screening Urine Sample Using Colour Test Method*

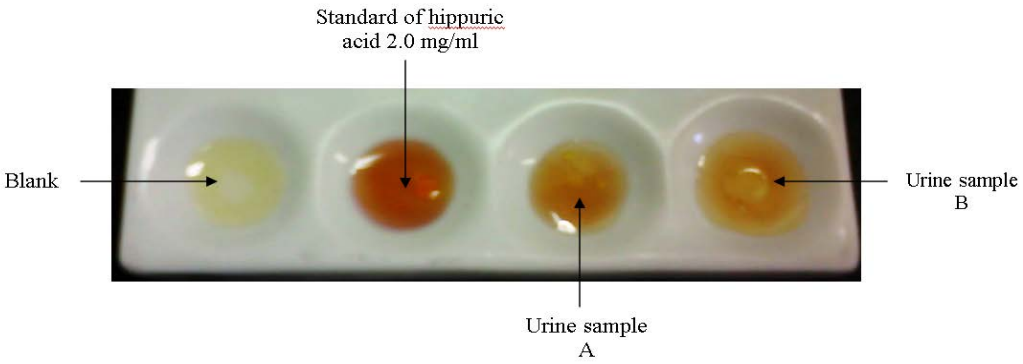

Figure 3.1.1.1 : Colour test for screening urine sample

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The colour test for screening urine sample has been shown at the Figure 3.1.1.1. When urine sample contained no hippuric acid, the mixture became colourless and transparent. The urine sample A and B illustrated light red colour similar to the colour of the hippuric acid standard. Thus proving that hippuric acid was present in this urine samples. As comparison, a standard hippuric acid of 2.0 mg/ml also shows the same colour development.

The reaction of the colour test method gave the red colour development. Figure 3.1.1.2 shows chemical equation between benzenesulphonychloride, pyridine and hippuric acid will take part when the chemicals mixed together. In that equation, the chloride will attacked the amine group or hydroxyl group to form ester and ether group. The chloride atom will be form hydrochloric acid. Pyridine is one of the indicators in this reaction similar to phenolphthalein. When the acidic reacted with the pyridine, the red colour developed in this reaction. The equations show that hydrochloric acid react with pyridine to form red colour. This is indicates the presence of hippuric acid in urine glue abuser.

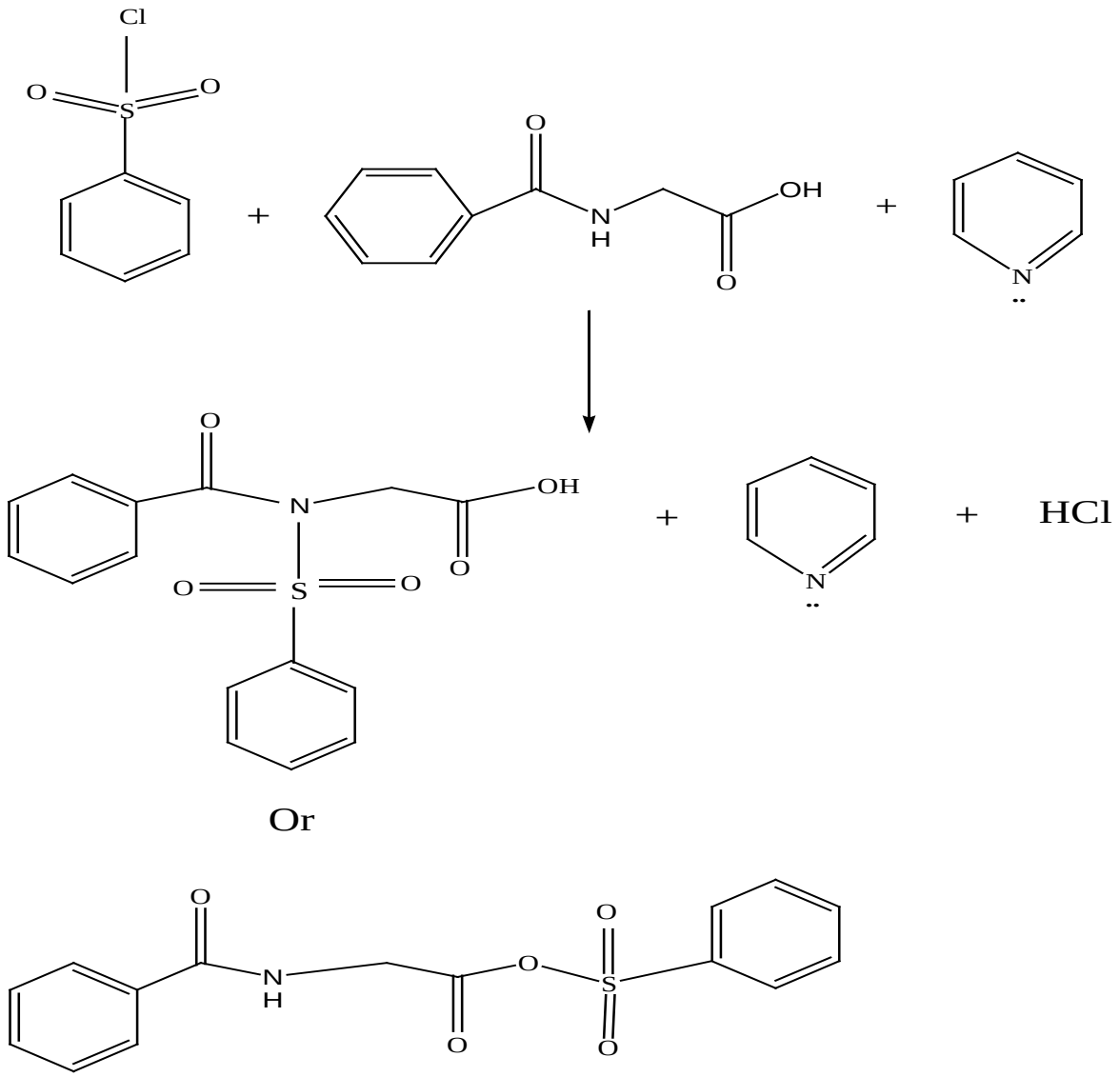

Figure 3.1.1.2 : Chemical equation of colour test method

iii. *Result of Screening Sample at Secondary School*

22 students were involved in the screening urine test at Seri Rahmat Secondary School, Johore Bahru. A number of 4 students gave positive results for hippuric acid in urine sample while the others gave negative results. At the same time, the National Anti Drug Agency was screening 5 drugs using dip strip kit to the same samples and none of the students are tested positive. The positive hippuric acid sample will be further analysing quantitatively using Ultra Violet Visible (UV-Vis) available in the lab. The colour reaction is shown in Figure 3.1.3. Even though, the colour test method is quite useful for qualitative analysis, the reddish colour of the reaction mixture faded gradually, so that semi quantification should be performed as soon as possible.

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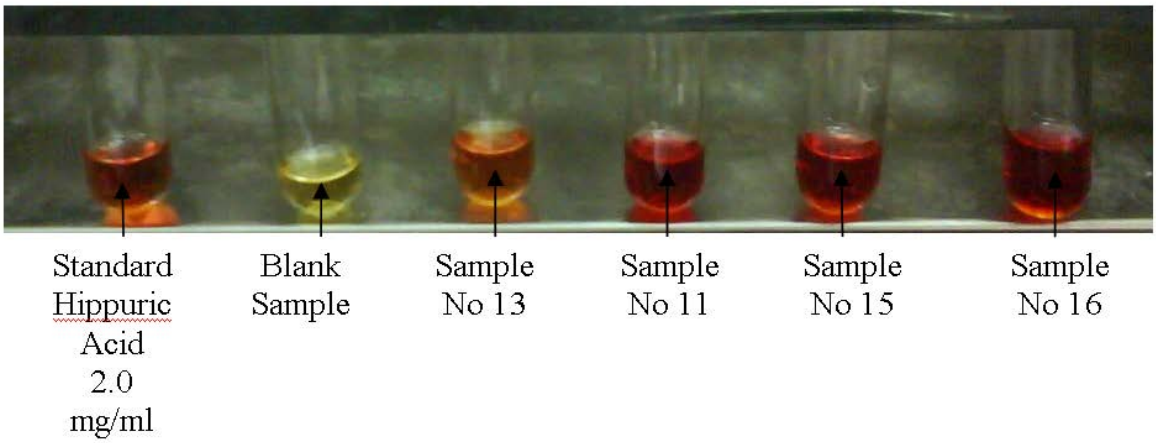

Figure 3.1.3 : Colour test for screening urine sample at secondary school

iv. *Result of Quantitatively of Hippuric Acid using Colour Test by Ultra Violet Visible*

Based from the screening urine test using color test method the result show four students gave the positive of existence of hippuric acid. From the calibration curve of hippuric acid using color test method the equation of y=0.4324x + 0.1293 the amount hippuric acid will be calculate with the R2=0.9933. Calculation amount of hippuric acid in urine has been show at Table 3.1.4.

Table 3.1.6 : Result of amount of hippuric acid using novel color reaction screening urine sample at secondary school.

Sample number	Amount of hippuric acid (mg/ml)
11	3.35
13	1.40
15	2.54
16	3.00

Based from the Manabu et al., (2005) stated that the amount of hippuric acid level more than 2.0 mg/ml indicates the sniffering of toluene with high probability and that a level from 1.0 to 2.0 mg/ml suggests the possibility of toluene abuse. Based from article, the normal human body will produce at least 0.10 mg/ml hippuric acid per day [14]. The result shows in the Table 3.1.6 three of the students have high probability of toluene abuse while other has possibility of toluene abuse.

IV. Conclusion

For screening urine of glue abuser color test method has been used to detect quantitatively hippuric acid with Ultra Violet Visible (UV-Vis) at wavelength 417 nm. It us calculate the intensity of red color. The coefficient value (R2) of determined by this method is 0.9933.

The method of color test are using pyridine and benzenesulphonyl chloride was yellow and became reddish by addition of distilled water has been reported by Manabu et al., (2005). It has been used in this research and has successfully resulted to detect glue sniffer easily using color reaction method with naked eye. In addition, this color test method has been used to screen urine sample among students at the secondary school in Johor Bahru.

Result from the screening at the secondary school show that from 22 students screening urine test, four of them give the positive existence of hippuric acid. Three of the students have high probability of toluene abuse while one of them gave possibility of toluene abuse.

In this case, the spectrophotometer method is thus useful for rapid screening quantitatively for hippuric acid in urine to glue sniffing and toluene abuser. However, the novel color reaction method would be quite useful for the screening of glue sniffing and toluene abuser qualitatively in way to help National Anti Drug Agency, Department of Education and Royal Police of Malaysia to prevent this problem become bigger.

V. Acknowledgment

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References Références Referencias

1. Hippuric Acid – Wikipedia the free encyclopedia. htm.
2. Serap A. A, Pembe O, Yusuf K., Huseyin.K, Suheyla.E, "Medicolegal Aspects of Blood-Urine Toluene and Urinary Ortho-Cresol concentration in

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Toluene Exposure". Journal Medical Science. 31: 415-419, 2001.
3. Arif, A. E.; and Navaratnam, V., eds. *Abuse of Volatile Solvents and Inhalants: Papers Presented at a WHO Advisory Meeting*. International Monograph Series No. 1. Penang, Malaysia: Drug Research Centre, Universiti Sains Malaysia, 1988. pp. 16-23.
4. Sharifah, M.T.F. "Report on the Inhalant Abuse Problem in Sarawak, East Malaysia." Paper presented at the Twelfth IFNGO Conference, Singapore, November 12-16, 1990.
5. Jasmine S, "Powerless to act on glue sniffing", New Straits Times, Monday, 6th June 2005.
6. Inhalant - Wikipedia, the free encyclopedia.htm "Electrochemical behaviour of electrodeposited Ni-WC composite coatings". *Surface & Coatings Technology.* Article in press.
7. International Programme on Chemical Safety (IPCS) (1986). Chemical. Environmental Health Criteria 52: Toluene. WHO. Geneva.
8. World Health Organisation (WHO) (2000). Air quality guidelines for Europe. WHO Regional Publications, European Series, No. 91. 2nd edition. WHO Regional Office for Europe. Copenhagen.
9. Department for Environment Food and Rural Affairs (DEFRA) and Environment Agency (EA) (2004). Contaminants in soil: Collation of toxicological data and intake values for humans. Toluene. R&D Publications TOX 14. Environment Agency. Bristol.
10. Carrie. E. Anderson, M. D. and Glenn A. Loomis, M.D, "Recognition and Prevention of Inhalant Abuse, St. Francis Hospital and Health Centers, Beech Grove, Indiana.
11. Yamazaki K, Tanaka E, Misawa S. "Urinary ortho-cresol concentrations as an indicator of toluene inhalation in glue sniffer". Journal Forensic Science. 32: 215-223 (1992).
12. Gaffney G. W, Schreier. K, Diferrante.N, Altman.K. "The quantitative determination of hippuric Acid". Journal Biochemistry. 206: 695-698 (1954).
13. Charles J. Umberger, Frank. F. Fiorese. Colorimetric method of hippuric acid. Journal of clinical chemistry. 72: 91-96 (1960).
14. Manabu Y., Atsushi A., Tomoaki M., Toshimitsu W. "Simple colorimetric semiquantitation method of hippuri acid in urine for demonstration of toluene abuse". Journal of Forensic Science and Legal Medicine. 7, 198-200 (2005).

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