Ortho-Cresol as Indicator for Toluene Exposure among Workers

Abd. Rahim Yacob\textsuperscript{1,2} and Nazirah Said\textsuperscript{2}

\textsuperscript{1,2}Chemistry Department, Faculty of Science, Universiti Teknologi Malaysia, 81310, Johor Bahru, Johor, Malaysia

\textbf{Abstract.} Toluene is a volatile organic solvent used by most industries and household items such as lacquers, paints, glue and nail polish. Recently, the negative health effects on exposure to toluene have attracted various studies. To combat the negative effects, American Conference of Governmental Industrial Hygienists (ACGIH) had lowered the occupational exposure limit from 100 ppm to 50 ppm of toluene vapour. Therefore, this could be translated into the need for a new, simple and more specific indicator test to monitor the degree of toluene exposure among workers. Thus, in this study, another metabolite of toluene, ortho-cresol was introduced. It was found that urinary ortho-cresol was more specific and sensitive in monitoring toluene exposure. Three samples out of six samples were found to have ortho-cresol concentrations between 3.00 ppm - 3.05 ppm. These concentrations are all above the permitted value of 3.00 ppm.

\textbf{Keywords:} Toluene, workers’ health, environment

1. Introduction

Different levels of exposure to toluene both acute and chronic have different effects on human especially in the central nervous system (CNS) which is the primary target of toluene in toxicity events. Acute inhalation of low or moderate level of toluene have reversible impairment to the CNS, narcosis and cardiac arrhythmia whilst higher level of toluene inhalation has caused CNS depression and death. In cases of toluene oral intake, severe depression of the CNS, constriction and necrosis of myocardial fibers, swollen liver, congestion and hemorrhage of the lungs, and tubular kidney necrosis have been accounted. Chronic exposure to toluene similar to occupational settings and in the cases of chronic inhalant abuse have been shown to cause drowsiness, ataxia, tremors, cerebral atrophy, nystagmus, impaired speech, hearing, and vision, irritation of the upper respiratory tract and eyes, sore throat, dizziness, headache, and difficulty with sleep [1], [2].

Better understanding about the importance of hygiene in occupational settings and the lowering of toluene contents in solvents has instigated the revision of safe exposure limit. The safe exposure limit has been reduced to half of the original set concentration, from 100 ppm to 50 ppm [3]. Most countries in the world have also followed the 50 ppm safety limit including Malaysia [4] hence, more specific biological indicator is needed to reflect the lower concentration of toluene limit to be detected [3].

Along with hippuric acid as its major metabolite, toluene is also converted into three other metabolites which are \textit{ortho-}, \textit{para-} and \textit{meta-}cresol [5]. The American Conference of Governmental Industrial Hygienists listed unmetabolized toluene in blood and hippuric acid and \textit{ortho-} cresol in urine as the biological markers of toluene exposure [6]. According to Stutaro \textit{et al.} (1989) [7], toluene is conjugated with glycine to hippuric acid before it is excreted via urine and only a very small amount of unmetabolized toluene. Thus, monitoring of exposure to toluene is done most commonly using hippuric acid’s amount present in urine [8].

The current study aims to utilize the superiority of \textit{ortho-}cresol as the marker for toluene exposure over hippuric acid which has been used traditionally as the indicator [7], [9]-[14].
2. Methodology

2.1. Chemicals and Samples

*Ortho*-cresol of GC grade was purchased from Merck-Schuchardt, toluene of AR grade was purchased from QREC Asia, Malaysia, methanol HPLC grade obtained from VS Chem House, Thailand, toluene of AR grade from Mallinckrodt Chemicals and Nitric acid 65% AR grade was obtained from BrightChem SDN. BHD, Malaysia.

Working *ortho*-cresol standard series of that range from 0.1 to 10 ppm were prepared using HPLC grade methanol as the solvent. These standards were used for constructing calibration graphs. The concentrations made were 0.1 ppm up to 1.0 ppm concentration *ortho*-cresol.

2.2. Instrumentation

The samples and standards used in the study were characterized and analyzed ultra violet visible (UV-Vis) brand Perkin Elmer. Ultra violet visible (UV-Vis) spectra were recorded at 350 to 400 nm to detect maximum absorbance of *ortho*-cresol and in urine.

2.3. Urine Samples

There were two types of urine samples collected which are control samples and exposed workers’ urine samples. The control samples are samples from workers from the same factory who were not exposed to toluene at any time during their working hours.

For samples of individuals exposed to toluene, the laboratory workers’ urine samples were collected. Urine samples were collected after an 8 hours work from six workers (5 males and 1 female) of a toluene manufacturing factory in Johor Bahru, Johor. These workers were engaged in quality testing the toluene that the company manufactured. None of the individuals wear mask during the toluene quality testing period and none of them ever take alcohol while 3 of them are smokers. These workers work 6 days per week, 8 hours per shift. Out of the 8 hours shift, a minimum of 2 hours must be spent quality testing the toluene. The urine samples collected were stored in a glass bottle with septum cap and were stored in the freezer (0°C) until analysis. Urine samples were collected were labeled S1, S2, S3, S4, S5 and S6 respectively.

![UV-Vis spectra](image)

Fig. 1: UV-Vis spectra of blank urine sample and sample spectra of urine spiked with *ortho*-cresol of concentrations 0.1 ppm, 0.2 ppm and 0.4 ppm

2.4. Colour Test Development

A colour test was developed during this study in order to determine the presence of *ortho*-cresol as a preliminary test in the urine sample before further characterization using UV-Vis technique. The colour produced at the end of the reaction as well as the shades or hues of the colour were identified and recorded.
The colours developed were determined by the examiner using naked eyes and tested at least three times respectively to confirm the reaction’s repeatability. One of the aims of the study was to develop colour test for ortho-cresol detection that uses reagents most commonly found in the laboratory which are easily accessible and environmental friendly.

### 3. Results and Discussions

Fig. 1 shows UV-Vis spectra of blank urine sample and spiked ortho-cresol 0.1 ppm to 0.4 ppm in urine. As shown, the maximum absorbance peak was found to be around 380 nm.

A series of ortho-cresol standard with concentrations ranging from 0.1 ppm to 1.0 ppm were subjected to UV-Vis analysis for the construction of a standard calibration graph. The following Fig. 2 shows the resulting calibration graph. The correlation of determination or \( R^2 \) was 0.9922 which was satisfactory and the equation was \( y = 2.245x \).

![Fig. 2: Calibration graph of ortho-cresol standard via UV-Vis.](image)

The reagent that met these criteria as well as that produces colour change when reacted with both ortho-cresol and control urine samples spiked with known concentration of ortho-cresol was nitric acid. The acid when poured into test tube containing known concentration of ortho-cresol and known concentration of ortho-cresol spiked in urine produced a colour reaction.

Creatinine correction of urinary ortho-cresol was not done on the samples. This is because there has been no international acceptance or need of creatinine adjustment as per reported by biological limits values recommended by American Conference of Governmental Industrial Hygienists [4].

Based on the colour test developed, the limit of detection of ortho-cresol in urine was 0.2 ppm. The following Fig. 3 shows the colour chart series developed from the tests ran.

![Fig. 3: Standard colour series chart for different ortho-cresol concentrations](image)

All six workers urine samples were analyzed using UV-Vis. The mean concentration of ortho-cresol in the samples was found to be 0.18 ppm and the highest concentration of ortho-cresol found was 3.05 ppm while the lowest was 0.41 ppm. The results are tabulated in Table 1.
Table 1: Ortho-cresol concentration recovered from workers urine sample analysis using UV-Vis

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ortho-cresol concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>3.20</td>
</tr>
<tr>
<td>S2</td>
<td>0.41</td>
</tr>
<tr>
<td>S3</td>
<td>3.05</td>
</tr>
<tr>
<td>S4</td>
<td>1.17</td>
</tr>
<tr>
<td>S5</td>
<td>0.84</td>
</tr>
<tr>
<td>S6</td>
<td>3.02</td>
</tr>
</tbody>
</table>

Fig. 4 shows the result for colour test done on workers urine samples. From the figure, samples 1, 3, 4, 5 and 6 showed colour reaction whereas sample 2 showed negative result. From the colour hue, it could be seen that S1 exhibited the darkest shade of colour which corresponds to the highest amount of ortho-cresol present amongst the workers followed by S6 and S3.

4. Conclusion

The colour test developed in the study showed its specificity to ortho-cresol as it reacted to even low amounts of ortho-cresol and did not produce positive results when tested with other metabolites of toluene especially the traditionally used hippuric acid.

5. Acknowledgement

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6. References


