Urinary Excretion of Cresol as an Indicator for Occupational Toluene Exposure

Koichi KONO,1* Yasuhisa YOSHIDA,1 Hiroshi YAMAGATA,1
Misuzu WATANABE,1 Yoshihiro TAKEDA,1
Michizo MURA0,1 Kazuhide DOI2
and Masahisa TAKATSU3)

1) Department of Hygiene and Public Health, Osaka Medical College,
Takatsuki, Osaka 569, Japan
2) Health Administration Room, Matsushita Electronics Co. Ltd.,
Takatsuki, Osaka 569, Japan
3) Forensic Science Laboratory, Hyogo Prefectural Police H.Q.,
Kobe 650, Japan

*Present Address: Dept. of Environmental Health, Fukui Medical
School, Matsuoka-cho, Fukui 910-11, Japan

(Received August 17, 1984 and in revised form November 8, 1984)

Abstract: Determination of urinary hippuric acid and phenolic compounds of
operators (n=33) working under relatively low concentration of toluene (average:
55 ppm) showed a good correlation between the hippuric acid and o- and p-cresol
(r=0.58 and 0.72, respectively). p-Cresol was always detectable in the urine in
contrast to o- and m-cresol, which were absent from the urine of 10% of the
operators exposed to toluene. In rats, experimentally injected with [methyl-14C]
toluene was metabolized into o-, p- and m-cresol. Furthermore, the level of
urinary cresol was not affected by intake of soft drinks, foods or medicaments,
which had pronounced effects on the urinary hippuric acid level.

The measurement of o- and p-cresol as well as hippuric acid concentration in
the urine was therefore thought to be a useful index for the occupational health
surveillance of toluene exposed workers.

Key words: Toluene exposure—Urinary cresol—Hippuric acid—Human urine—
Occupational health surveillance

INTRODUCTION

Toluene is a widely used industrial solvent. In the body it is metabolized mainly
into benzoic acid, which is then conjugated with glycine and excreted via the
urine as hippuric acid. Although the concentration of urinary hippuric acid is
a useful indicator of toluene exposure,1-4) it is sometimes altered by the intake of
medicaments and foodstuffs which contain various amounts of benzoic acid or its precursors such as quinic acid.\textsuperscript{4,5)} Formation of \textit{o-}, \textit{p-} and \textit{m-}cresol after exposure to toluene was reported by several investigators.\textsuperscript{6-10)} Angerer\textsuperscript{6) examined a group of print workers exposed to toluene, and suggested that, besides hippuric acid, a small amount of \textit{o-}cresol was also synthesized from toluene. Pfäffli\textsuperscript{7)} \textit{et al.} determining the urinary \textit{o-}cresol under varying toluene vapour exposure levels showed a linear relationship between the metabolite and the inhaled solvent.

In this paper, phenolic compounds in the urine of rats after experimentally injected \textit{[methyl-\textsuperscript{14}C] toluene} was measured to investigate the mechanism of toluene metabolism. We also examined the relationship between urinary phenolic compounds and hippuric acid at relatively low toluene exposure levels in order to clarify the usefulness of urinary cresol determination, in addition to hippuric acid, for biological monitoring and occupational health surveillance of toluene workers.

**Materials and Methods**

1. **Subject:**

For the determination of urinary hippuric acid and cresol after toluene exposure, urine specimen of subjects (\textit{n=33}) working in a relatively low toluene concentration environment (40 to 80 ppm, mean 55 ppm) for 5 to 6 hours per day in the electronics industry were studied. The workers were engaged in the washing process of glass bulbs for TV picture tubes, where toluene containing not more than 0.001\% benzene was the sole solvent. Spot urine specimens were taken four times (8:00, 11:30, 13:00 and 16:00 hours) during a work day, either on Thursday or Friday.

For the investigation of the effects of medicament and food intake on the urinary excretion of hippuric acid and phenolic compounds, 0.2 g sodium benzoate or 350 ml soft drinks (\textit{e.g.} cola) which contain sodium benzoate as a preservative were administered to healthy male volunteers aged 20 to 35 (\textit{n=4}).

2. **Animal experiments:**

Wistar male rats weighing about 250 g were starved for one day before toluene injection in order to exclude the effect of foods on hippuric acid formation. \textit{[Methyl-\textsuperscript{14}C] toluene} (3.7 \textmu Ci/0.12 ml/rat) was administered by intraperitoneal injection and 15 ml of water was directly infused into the stomach of each animals through a catheter in order to increase the amount of urine. Control rats received only water. Urine was collected at 24 hr intervals for three days.

3. **Analytical methods:**

\textit{Reagents}.
All solvents and chemicals used for analysis were of reagent grade. [Methyl-\textsuperscript{14}C] toluene (specific activity 30.2 mCi/mmol) was supplied by Radiochemical Centre, Amersham, U.K. and diluted with unlabeled toluene to a specific activity of 3.25 mCi/mmol.

**Detection of metabolites in urine.**

Urinary hippuric acid was determined by the direct colorimetric method of Tomokuni and Ogata.\textsuperscript{11}) The obtained concentrations were corrected using a specific gravity of 1.024.

\textit{o-}, \textit{p-} and \textit{m}-cresol and phenol were determined by gas chromatography. Urine samples were prepared as described by Kawai and Horiguchi.\textsuperscript{12}) A 2 ml conc-HCl was added to a 5-ml urine specimen and the solution was hydrolysed at 100°C in a water bath for 1 hour. After 1 ml of isopropyl ether containing 50 µg of 2,4-xylenol as an internal standard was added, the sample was shaken vigorously on a Vortex mixer and centrifuged at 3000 rpm for 10 minutes. The clear supernatant solution was examined by gas chromatography. For the measurement of radioactive metabolites, urine was extracted three times with isopropyl ether and all extracts were combined and concentrated to the detection limit by evaporation at 50°C.

**Gas chromatography.**

Analysis was carried out by a Shimadzu Model 6A gas chromatographic apparatus. The analytical column was a glass tube of 300 cm in length and 3 mm in inner diameter packed with FAP-S 60–80 mesh on Chromosorb W (AW). The oven temperature was maintained at 160°C, and both the injection and detector part (hydrogen flame ionizer) at 220°C. The data were processed by a microcomputer having the parameters pre-set (Shimadzu Chromatopack) Model C-RIB). The detection limit for cresols under our chromatographic method was 20 µg/l. Labeled metabolites were determined by a Shimadzu Model 7A gas

**Diagram:**

1. Urine sample
2. Added conc-HCl
3. Hydrolyzed at 100°C for 1 hr.
4. Cooled to room temp.
5. Extracted with isopropyl ether (1 ml) containing 50 µl of 2,4-xylenol as internal standard
6. Centrifuged at 3000 rpm for 10 min.
7. Organic phase
8. Concentrated by evaporator
9. Separated & trapped by gas chromatography equipped with fraction collector
10. Added scintillator
11. Counted the radioactivities by liquid scintillation counter

**Fig. 1. Protocol for the determination of radioactive phenolic compounds in urine**
chromatograph and collected fractionally in impingers containing 10 ml of ethyl ether in an ice bath equipped with a Shimadzu Model APP-5 fraction collector. After the solvent in each fraction was evaporated to about 0.5 ml, 10 ml of Amersham ACS II scintillation cocktail was added. The radioactivity was determined using a Packard Model 300C liquid scintillation counter. The detailed protocol for the determination of radioactive metabolites is shown in Fig. 1.

**RESULTS**

Table 1 shows the mean concentrations of urinary metabolites from toluene exposed workers (n=33) collected at different hours during a work day. The excretion of hippuric acid into the urine increased rapidly after the exposure to toluene and reached to a maximum level being three times of the pre-exposure value at the end of a working day (16:00 hrs). As described in our previous paper, the normal value of hippuric acid in the urine of non-toluene exposed workers was 0.34±0.37 g/l (arithmetic mean±S.D.). In the present study, the pre-exposure level of urinary hippuric acid was higher (p<0.01) than the normal value, which suggested that toluene detoxication in the body lasted over at least one night. p-Cresol was a major component of the phenolic compounds, while the levels of o- and m-cresol detected were very low and they were undetectable in 15 and 18 out of 132 samples. The urinary excretion of o- and p-cresol paralleled with that of hippuric acid and reached to maximum at 16:00 hours in a work day. At the time, the concentration of o-cresol was about two and half times and that of p-cresol was one and half times larger than pre-exposure values. These substances were related to toluene inhalation and the values of the biological half time of o- and p-cresol were similar to that of hippuric acid. The urinary excretion of m-cresol did not parallel with that of hippuric acid and of other cresol isomers, but the concentration at 16:00 hours was considerably higher than that at 8:00 hours. Although a considerable amount of phenol was also excreted as indicated in Table 1, the level showed no change during a work day and seemed to be due to normal urinary excretion as was found in healthy control subjects. Table 2 shows the correlation coefficient and regression equation

<table>
<thead>
<tr>
<th>Time</th>
<th>8:00 (before expo.)</th>
<th>11:30</th>
<th>13:00</th>
<th>16:00</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA (g/l)</td>
<td>0.73±0.46</td>
<td>1.67±1.26</td>
<td>2.00±1.37</td>
<td>2.16±1.45</td>
</tr>
<tr>
<td>o-cresol (mg/l)</td>
<td>0.25±0.29</td>
<td>0.34±0.26</td>
<td>0.59±0.42</td>
<td>0.63±0.50</td>
</tr>
<tr>
<td>p-cresol (mg/l)</td>
<td>20.78±21.09</td>
<td>22.44±22.56</td>
<td>26.86±25.37</td>
<td>27.21±23.64</td>
</tr>
<tr>
<td>m-cresol (mg/l)</td>
<td>0.08±0.12</td>
<td>0.08±0.11</td>
<td>0.12±0.11</td>
<td>0.13±0.09</td>
</tr>
<tr>
<td>phenol (mg/l)</td>
<td>6.38±6.09</td>
<td>4.80±4.48</td>
<td>5.58±5.59</td>
<td>4.29±4.71</td>
</tr>
</tbody>
</table>
between the levels of excretion of hippuric acid and those of phenolic compounds. The concentrations of \(o\)- and \(p\)-cresol in the urine of toluene exposed workers showed statistically significant correlations with that of hippuric acid \((r=0.58\) and \(0.72\), respectively). The correlation coefficient between hippuric acid and \(m\)-cresol was not statistically significant \((r=0.28)\), since the concentration and the increase of urinary \(m\)-cresol during a work day were very small and this substance was not detectable in about 10% of the samples. No correlation was found between hippuric acid and phenol. These results showed that not only

<table>
<thead>
<tr>
<th>(a)</th>
<th>(b)</th>
<th>(r)</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(o)-cresol</td>
<td>117</td>
<td>0.07X + 0.34</td>
<td>0.58</td>
</tr>
<tr>
<td>(p)-cresol</td>
<td>132</td>
<td>9.65X + 8.32</td>
<td>0.72</td>
</tr>
<tr>
<td>(m)-cresol</td>
<td>114</td>
<td>0.03X + 0.05</td>
<td>0.28</td>
</tr>
<tr>
<td>phenol</td>
<td>132</td>
<td>-0.93X + 6.77</td>
<td>-0.21</td>
</tr>
</tbody>
</table>

\(Y\): Concentration of phenolic metabolites in mg/l
\(X\): Concentration of hippuric acid in g/l
\(r\): Coefficient of correlation
\(p\): Level of significance

**Fig. 2. Gas chromatogram of the urine extract in toluene administered rat**
hippuric acid but also \( o \)- and \( p \)-cresol were useful indexes of toluene exposure.

A gas chromatogram (Fig. 2) of radioactive urine extract from a \([\text{methyl-}^{14}\text{C}]\)Table 3. Radioactivity in the urinary phenolic compounds fractionated by gas chromatography

<table>
<thead>
<tr>
<th>Substance</th>
<th>Peak area</th>
<th>Radioactivity (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( o )-cresol</td>
<td>90612</td>
<td>1946</td>
</tr>
<tr>
<td>( p )-cresol</td>
<td>663974</td>
<td>47324</td>
</tr>
<tr>
<td>( m )-cresol</td>
<td>5067</td>
<td>188</td>
</tr>
<tr>
<td>phenol</td>
<td>216912</td>
<td>15</td>
</tr>
</tbody>
</table>

![Graph](image)

Fig. 3. Changes in urinary metabolites concentration in 4 healthy subjects after sodium benzoate (0.2 g) administration
toluene administered rat, revealed an initial large peak followed by a tailing of the solvent. However, the peaks of internal standard (2,4-xylene) and phenolic compounds were separated under the present chromatographic condition.

As shown in Table 3, of the urinary phenolic compounds o- and p-cresol were strongly labeled indicating them to be the major metabolites of toluene besides hippuric acid. Although the radioactivity in the m-cresol fraction was relatively weak, it suggests this substance also to be a metabolite of toluene. In contrast to the radioactivity of cresol isomers, its level in phenol was within the background range, which means that phenol is not a biological metabolite of toluene.

Intake of 0.2 g sodium benzoate (Fig. 3) produced a relatively sharp increase in the concentration of hippuric acid in the urine, reaching a maximum concentration after 30 min to 1 hour. Compared with the high level excretion of hippuric acid in the urine, the concentrations of urinary cresol and phenol were not affected by the intake of food or drink.

**DISCUSSION**

When a worker is exposed to toluene, about 20% of the absorbed solvent is excreted through the lungs in unchanged form. The major portion, approximately 70 to 80% of toluene, is oxidized, conjugated and then excreted into the urine as hippuric acid. This fact is generally accepted as a proof of toluene exposure. Hippuric acid is also found in the normal urine being derived from natural foods, foodstuffs or medicaments containing benzoic acid and its precursors. Coffee beans and some kinds of vegetables and fruits contain benzoic acid and quinic acid. Japan’s Food Sanitation Act allows the use of benzoic acid as a preservative for some soft drinks. In our study, the intake of 0.2 g of sodium benzoate or 350 ml of soft drink induced increase of urinary hippuric acid reaching over 3.5 g/l. This value is equal to the average concentration in workers exposed to 100 ppm of toluene, which corresponds to the maximum allowable concentration suggested by the American Conference of Governmental Industrial Hygienist (ACGIH) and the Japan Association of Industrial Health (JAIH). Therefore, for evaluation of toluene exposure by determination of hippuric acid in spot urine, the intake of these foods should be controlled at the time of urine sampling.

The excretion of o-, p- and m-cresol in the urine of toluene exposed workers has recently been discussed. In our study, the amount of o- and m-cresol were very small probably because the workers were exposed to relatively low concentrations of toluene. A linear correlation, however, was found between the concentration of urinary hippuric acid and that of o- or p-cresol in these specimens. Furthermore, the amount of o- and p-cresol in the urine was not scarcely affected by the intake of soft drinks or medicaments which had pronounced effects on the level of urinary hippuric acid. It is commonly accepted that p-cresol is also a normal urinary constituent, and its relatively large fluctuations in its urine level
are caused by individual differences in the characteristics of cresol metabolism of which control is difficult. In spite of this fact, \( p \)-cresol concentration in urine of the workers reflected faithfully their toluene exposure even at low concentrations as described above.

Daly et al.\textsuperscript{15} mentioned that \( m \)-cresol seemed to be an unlikely intermediate as the result of toluene hydroxylation in the process of opening of toluene oxidation. In our study, the radioactivity in the urinary cresol isomers of [methyl-\( ^{14} \)C] toluene administered rat suggested that \( o \)-, \( p \)- and \( m \)-cresol were the metabolite of toluene. However, our data is too limited to be conclusive.

The results presented here indicate the measurement of urinary \( o \)- and \( p \)-cresol in addition to hippuric acid to be a useful indicator for the occupational health care of toluene exposed workers.

\textbf{References}