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Urinary hippuric acid concentration after occupational exposure to toluene

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ABSTRACT The results of industrial investigations have shown a correlation between the rate of hippuric acid excretion in a single urine sample collected after daily occupational exposure and the amount of toluene absorbed. The rate of hippuric acid excretion and the average concentration of toluene vapour during exposure time were also related. The quantitative range of the test has been limited to amounts exceeding 425 mg of toluene and concentrations exceeding 69 ppm of toluene in the air because of the physiological presence of hippuric acid in urine. The rate of hippuric acid excretion in urine depends on diuresis and is constant for urinary fractions with diuresis of 30 ml/h. The physiological excretion rate was 20 mg/h with a standard deviation ± 4·3 mg/h, and maximal physiological level 33 mg/h.

Biochemical exposure tests are of considerable value in the diagnosis of occupational and environmental poisoning. Exposure tests, as opposed to an index based upon the concentration of toxic substances in the air, allow for a much more accurate estimation of the degree of occupational exposure.

Srobova and Teisinger (1953) proposed an exposure test for toluene which was based on the concentration of benzoic acid in urine collected over 24 hours. The test was modified by Piotrowski (1967) who accepted the excretion rate measured at the end of daily exposure as the excretion unit. He defined the degree of exposure to toluene on the basis of the amount of toluene absorbed. The method of determining benzoic acid (Teisinger et al., 1956) depends on the hydrolysis of hippuric acid and small amounts of benzoyl glucuronic acid as toluene metabolites (Srobova and Teisinger, 1953; Williams, 1959). In addition, the method of determination is time-consuming and expensive, and therefore impracticable for serial testing; nevertheless much of the data established by Piotrowski (1967) are still valid and important.

According to El Masry et al. (1956) hippuric acid is the main toluene metabolite excreted in urine. Pagnotto and Lieberman (1967), Ogata et al. (1968) and Ikeda and Ohitsuji (1969) have reported a correlation between the concentration of toluene and the amount of hippuric acid in urine, and suggest that estimation of exposure to toluene should be based on the concentration of hippuric acid in urine. Ogata et al. (1970) have established under experimental conditions the relation between occupational exposure (expressed as the ratio between vapour densities and exposure time) and the concentration of hippuric acid in urine collected over 24 hours. They have also defined the screening levels of concentration and excretion rate of hippuric acid, corresponding to the American standard (TLV).

The aim of our investigation was to define the relationship between the average concentration of toluene vapour and the amount of toluene absorbed by the body on the one hand, and the value determining the excretion of hippuric acid, the main metabolite of toluene, in urine on the other. The exposure test was based on the measurement of the hippuric acid excretion rate in urine collected at the end of occupational exposure only. Physiological levels were studied separately. From the toxicological point of view the problem is not so much the determination of the hippuric acid level in urine, as the determination of the increase in the metabolite level caused by absorption of toluene vapour, in other words, the determination of a value above which the absorption of toluene vapour may be assumed to have occurred.

The investigations have been conducted under industrial conditions. This was prompted by differences between results obtained during industrial
exposure and those obtained under experimental conditions (Pagnotto and Lieberman, 1967; Ikeda and Ohtsuji, 1969; Ogata et al., 1970).

Materials and methods

Urine samples were collected from 236 workers divided into three groups: from 52 workers occupationally exposed to toluene every four hours after daily exposure to a concentration range of 30-180 ppm, and two control groups consisting of 101 subjects not exposed occupationally to toluene, and 82 subjects occupationally exposed to toluene but not exposed on the study day. Urine was collected at about noon. The concentration of hippuric acid in urine was determined and then the rate of metabolite excretion was calculated. Air samples were collected in the workroom close to workers' faces at short intervals (approximately every hour), and the concentration of toluene vapour was analysed for each conventionally limited exposure zone. The average concentration of toluene vapour was then established. Measurements were then taken with the object of achieving an accurate determination of exposure time in a given zone of toxic exposure. The amount of toluene absorbed by inhalation was estimated for successive time units, assuming lung ventilation of 0.75 m³/h and retention of toluene vapour in the lungs of 72% in the first two hours and 57% afterwards. The data are cited from Piotrowski (1967) who described the quantitative absorption of toluene by humans based on benzoic acid determination in urine. The amount of toluene absorbed was calculated on the basis of lung ventilation, retention of toluene vapour, the mean concentration of toluene in the zone analysed and the duration of individual exposure. This amount was characterised by a given excretion rate of hippuric acid measured in the urine sample of each worker. These data were correlated and presented as dependent on the rate of hippuric acid excretion in the four-hour urine sample collected immediately after the last four hours of exposure to toluene for the calculated amount of toluene absorbed. Similarly, the measured mean value of toluene concentration in the air of the analysed zone and the excretion rate of hippuric acid in such a four-hour urine sample were correlated. However, only 34 of the urine samples collected from 52 workers which were analysed were taken into account. The data from the other 18 workers were omitted because, as is mentioned below, statistically highly significant variations in the physiological level of hippuric acid excretion were found in urine samples from subjects with low diuresis. In the reported results the diuresis of the 18 workers omitted was below 30 ml/h.

The workers were doing light work, and toluene did not come into contact with the skin of their hands. The workers' history did not suggest any potential drug influence.

Determination of Toluene Concentration in the Air

Determination of toluene vapour in the air was carried out in accordance with the Polish standard PN 67/Z-04090, describing the method of determination of atmospherc toluene that must be used in Polish industry. It consists of the nitration of toluene to 2,4,6-trinitrotoluene and its subsequent reaction with ethyl alcohol and potassium hydroxide. The absorbance of the coloured solution is estimated at $\lambda = 520$ nm.

Urine Analysis

Determination of hippuric acid in urine was carried out according to the method of Burkiewicz and Zielińska (1972). The method consists of extraction of urine with chloroform and the reaction of hippuric acid in an anhydrous environment with acetic anhydride in the presence of pyridine. For this determination 0.4 ml of urine was added to 0.05 ml of concentrated H$_2$SO$_4$ and extracted with 10 ml of chloroform. Seven ml of pyridine: acetic anhydride mixture (5:2 v/v) was added to 4 ml of the chloroform layer and stirred for 90 min at room temperature. The absorbance of the coloured complex was measured at 430 nm. Hippuric acid concentrations were based on a urine specific gravity of 1.024. The simple method uses generally available and inexpensive reagents and can be used for serial testing, which makes the test widely applicable.

Specific gravity was determined with a urinometer; creatinine concentration was estimated by the well-known Folin-Wu method.

Results and discussion

The physiological level was studied in two stages, from May to July and from August to October. Teisinger and Srobova (1954) and Piotrowski (1967) consider that variations in the physiological level of metabolites depend on fruit and vegetable consumption. The values of the physiological level of hippuric acid determined in the course of the investigations are shown in Tables 1 and 2. Variations in the physiological rate of hippuric acid urinary excretion of the groups occupationally exposed and not exposed to toluene in different seasons of the year are not statistically significant ($p > 0.1$). Statistically highly significant variations in the physiological rate of hippuric acid excretion have been noted in urine...
The physiological level of hippuric acid in urine

Table 1

<table>
<thead>
<tr>
<th>Study periods and Group</th>
<th>May-July</th>
<th>August-October</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>No. of subjects</td>
<td>70</td>
<td>40</td>
</tr>
<tr>
<td>Concentration of creatinine (mg/ml)</td>
<td>0.154-2.752 (0.843)</td>
<td>0.181-1.220 (1.171)</td>
</tr>
<tr>
<td>Hippuric acid concentration (mg/ml)</td>
<td>0.034-0.740 (0.306)</td>
<td>0.090-0.790 (0.447)</td>
</tr>
<tr>
<td>Hippuric acid concentration (mg/ml corrected for specific gravity 1.024)</td>
<td>0.052-0.840 (0.402)</td>
<td>0.180-0.705 (0.415)</td>
</tr>
<tr>
<td>Excretion rate (mg/h)</td>
<td>14.60 ± 3.8</td>
<td>17.63 ± 4.3</td>
</tr>
</tbody>
</table>

Group A; subjects not exposed occupationally to toluene.
Group B; subjects exposed occupationally but not exposed to toluene on the study day and the preceding day.
Means in parentheses.

The rate of hippuric acid excretion in urine in relation to diuresis

Table 2

<table>
<thead>
<tr>
<th>Diuresis (ml/h)</th>
<th>May-July</th>
<th>August-October</th>
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</thead>
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<table>
<thead>
<tr>
<th>Diuresis (ml/h)</th>
<th>Group A</th>
<th>Group B</th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n)</td>
<td>Hippuric acid excretion (mg/h)</td>
<td>Hippuric acid excretion (mg/h)</td>
<td>Hippuric acid excretion (mg/h)</td>
<td>Hippuric acid excretion (mg/h)</td>
</tr>
<tr>
<td>&lt; 30</td>
<td>20</td>
<td>7.48 ± 3.2</td>
<td>8</td>
<td>12.30 ± 2.8</td>
</tr>
<tr>
<td>31-50</td>
<td>22</td>
<td>18.47 ± 2.7</td>
<td>14</td>
<td>20.51 ± 3.4</td>
</tr>
<tr>
<td>51-80</td>
<td>15</td>
<td>22.70 ± 3.4</td>
<td>12</td>
<td>21.92 ± 2.6</td>
</tr>
<tr>
<td>80&lt;</td>
<td>13</td>
<td>20.92 ± 3.8</td>
<td>6</td>
<td>23.00 ± 4.1</td>
</tr>
</tbody>
</table>

Group A; subjects not exposed occupationally to toluene.
Group B; subjects exposed occupationally but not exposed to toluene on the study day and the preceding day.

Samples with diuresis below or above 30 ml/h (0.005 > p > 0.001). The values of the physiological level of hippuric acid in urine tend to vary considerably (Table 1). The variation can be observed in the same subjects on different days and also in successive urine samples collected over 24 hours. The accepted physiological excretion rate was 20mg/h with the average standard deviation ±4.3 mg/h. The maximum physiological excretion rate may reach the value of 33 mg/h assuming three standard deviations. It can be assumed that absorption of toluene vapour occurs when this value is exceeded.

Analysis of successive urine samples collected over 24 hours showed an increase in the level of hippuric acid. Concentrations were above the physiological level before work, up to the maximum value at the end of a working day; in addition, the concentration decreased to the physiological level 14 hours after the exposure time. The maximum excretion period occurs in the final phase of daily exposure; hippuric acid concentration in urine in the last four hours of daily exposure is about 2.5 times higher than the concentration in the total 24-hour urine sample. The data reported above point to the necessity of devising an exposure test based on the determination of hippuric acid excretion rate in one sample of urine collected at the maximum excretion time, which is advantageous both for practical reasons and for accuracy and interpretation of results (the effect of physiological level variations is then lowest).

Experimental data showed that a four-hour specimen collected immediately after the last four hours of exposure should be recommended as the most appropriate for estimating the extent of the exposure.

A statistical correlation has been established between the hippuric acid excretion rate (corrected for urine of specific gravity 1.024) plotted against the amount of toluene absorbed have been shown to fall on the straight regression line described by equation (1):

\[ y = 14.7 + 0.151x \]

where: \( y \) = the rate of hippuric acid excretion in mg/h in the four-hour urine sample collected after exposure

\( x \) = amount of toluene (in mg) absorbed by inhalation during exposure.
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**Fig. 1** The relationship between the amount of toluene and the rate of hippuric acid excretion in a four-hour urine sample collected at the end of eight hours' exposure.

This correlation is graphically represented in Fig. 1, which shows that the correlation coefficient \( r = 0.97 \) (\( p > 0.001 \)) and the dispersion from straight regression is \( S_{y|x} = \pm 16.62 \).

As the test accuracy (the standard deviation) is ±85 mg assuming a standard deviation value ±20% as desirable for toxicological surveys, the quantitative range of the test may be limited to amounts exceeding 425 mg toluene. In industrial conditions this corresponds to a concentration of about 30-85 ppm toluene vapour in the air.

The statistical calculations have established the correlation between the rate of hippuric acid excretion in urine collected four hours after exposure time, and the average concentration of toluene vapour in the air. The correlation is represented in equation (2):

\[
y = 64.7 + 0.932 x \tag{2}
\]

where: \( y \) = the rate of hippuric acid excretion in mg/h in urine collected four hours after daily exposure

\( x \) = the average concentration of toluene vapour in ppm in the air during exposure.

**Fig. 2** The relationship between the mean toluene level in the air and the rate of hippuric acid excretion in a four-hour urine sample collected at the end of eight hours' exposure.

This correlation is graphically represented in Fig. 2 (\( r = 0.67; p > 0.001 \)). Dispersion round the straight regression line is \( S_{y|x} = 55.46 \). The accuracy of the test is 13.8 ppm. Assuming a deviation value ±20% as desirable in toxicological determinations, the quantitative range of the test may be limited to concentrations exceeding 69.1 ppm.

Considering the high rate of metabolic processes of toluene (excretion constant \( k = 0.36 \) (Piotrowski, 1967)) any influence of the previous day's exposure on the results can be excluded. Therefore, the results must reflect exclusively the degree of exposure on the days the sample was collected.

**References**


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