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Urinary *o*-cresol in toluene exposure

by PIRKKO PFÄFFLI, M.Sc., HEIKKI SAVOLAINEN, M.D., PIRKKO-LIISA KALLIOMÄKI, M.D., L.Sc. (Eng.), and PENTTI KALLIOKOSKI, Ph.D. (Eng.)¹

PFÄFFLI, P., SAVOLAINEN, H., KALLIOMÄKI, P.-L. and KALLIOKOSKI, P. Urinary *o*-cresol in toluene exposure. *Scand. j. work environ. & health* 5 (1979) 286—289. The determination of urinary *o*-cresol in varying exposure to toluene vapor showed a linear relationship between the metabolite and the inhaled solvent. The analytical method involves acid hydrolysis of 20 ml of urine with subsequent extraction of the phenolic compounds in dichloromethane, followed by gas chromatographic analysis. The lowest limit of detection lies at 0.5 $\mu\text{mol/l}$, whereas the practical limit as an exposure test is at 2.5 $\mu\text{mol/l}$ [corresponding to 0.2 $\mu\text{mol/l}$ (5 ppm) of toluene vapor]. The test may also have indirect toxicologic significance, as the underlying initial arene oxides which produce the cresolic compounds are involved in toluene toxicity.

Key words: biological exposure test, toluene exposure, urinary *o*-cresol.

Toluene is a common constituent of a wide range of industrial products. It is also used as a general solvent in work extending from the printing industry to domestic uses.

Toluene is actively metabolized by the human body to produce mainly benzoic acid, which is conjugated with glycine to produce hippuric acid (5). Although present in abundant quantities in the urine of toluene-exposed persons, hippuric acid lacks the necessary specificity as a biological exposure test because food contains varying amounts of benzoic acid as a genuine or added constituent and gives rise to hippuric acid in the urine of the general population (3).

A minor fraction of inhaled toluene vapor is oxidized at the aromatic ring.

This pathway produces initial arene oxides which rearrange spontaneously to *o*- or *p*-cresol (8). Consequently, these phenolic compounds have been identified in the urine of experimentally exposed animals (2) or in human toluene exposure (1). However, the same drawback applies to the para substituted isomer as to hippuric acid (1, 2), whereas *o*-cresol is not a major constituent of normal urine.

In this paper we have described a gas chromatographic method for the determination of urinary *o*-cresol, and we show its relation to toluene exposure. We have also examined its disappearance after the end of exposure in order to determine the optimal time for its measurement as a biological exposure test.

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MATERIALS AND METHODS

Twenty-one male rotogravure printers 36.7 (SD $\pm$ 7.9) years of age, with 18.2

(SD $\pm$ 9.4) years in their current occupation, participated in this study. They worked in three different printing plants where toluene was the sole solvent used in the printing process.

The personal toluene exposure of the printers was evaluated by the charcoal tube sampling method (7). The air samples were collected hourly during a whole workday, either on Thursday or Friday. The personal toluene exposures of the printers were calculated as the time-weighted average concentrations for an 8-h workday, and they varied from 0.3 to 4.5 $\mu\text{mol/l}$ (7 to 112 ppm).

Urine specimens were taken immediately after the workshift. In another experiment, six printers with an average toluene exposure of $1.9 \pm 0.2 \mu\text{mol/l}$ also gave urine samples for 8 h after the end of the shift and on the next morning. Similar samples were also obtained from four other printers with an average toluene exposure of $3.7 \pm 0.4 \mu\text{mol/l}$. The urinary *o*-cresol concentrations were corrected for excretion volume by creatinine determination. The samples were stored at -25°C until analysis.

Urinary *o*-cresol was assayed after acid hydrolysis by capillary gas chromatography. The hydrolysis (2) was carried out in approximately 1.6 M sulfuric acid by the refluxing of a 20-ml portion of a urine sample and 2 ml of concentrated sulfuric acid in a boiling water bath. The refluxing time was 1 h. An internal standard, 20 μg of 2,4-dimethylphenol, was added to the samples to correct the subsequent loss of *o*-cresol. The hydrolyzed urine samples were extracted three times with 25-ml portions of dichloromethane, and the combined extracts were shaken three times with 50 ml of a 5 % sodium bicarbonate water solution to remove acids. The dichloromethane solution was dried with sodium sulfate, evaporated carefully to dryness in a rotavapor (Büchi Rotavapor "R", Switzerland), and dissolved without delay in 1 ml of dichloromethane. The dichloromethane solutions were analyzed by gas chromatography (fig. 1).

The phenolic compounds were identified in a comparison with reference substances in two separate gas chromatographic columns, i.e., a packed QF1 column (3 % on Chromosorb W AW, length 2 m, inner dia-

meter 2 mm, temperature 80°C) and an OV 225/151 glass capillary column. The procedure for the capillary gas chromatography was as follows: gas chromatograph: Perkin-Elmer, Sigma 3, equipped with a flame ionization detector; column: glass capillary, length 29 m, inner diameter 0.273 mm, liquid phase OV 225/151; column temperature: programmed after a 1-min isothermal operation from 40°C to 135°C with a rate of 10°C/min , then 15 min isothermal; carrier gas: helium, flow 1 ml/min; injection: 1 μl , split ratio 1 to 10.

The response of the flame ionization detector for 1 ng of *o*-cresol caused the peak height of 1 cm when the attenuator of the gas chromatograph was 1×2 .

Fig. 1. Capillary gas chromatogram of urinary phenols. The retention times (min) are in parentheses. (1 = phenol (11.4 min), 2 = *o*-cresol (12.0 min), 3 = *m*- and *p*-cresol (13.1 min), 4 = 2,4-dimethylphenol (13.9 min) as the internal standard, and S = solvent)

Fig. 2. Linear relationship of the time-weighted average of toluene concentrations in the air and the urinary *o*-cresol concentrations as collected immediately after the end of exposure. Coefficient of correlation is indicated by *r*.

RESULTS

The present chromatographic technique yielded a satisfactory separation of the phenolic compounds in the urine. Especially *o*-cresol appeared as a distinct separate peak (fig. 1). The analytical procedure allowed the detection of *o*-cresol at a concentration of 0.5 $\mu\text{mol/l}$. Experiments with added *o*-cresol showed a 99% recovery at a concentration of 10 $\mu\text{mol/l}$ ($N = 9$), whereas the precision was better than 0.02 (coefficient of variation).

The output of *o*-cresol in the urine of the toluene-exposed printers was linearly proportional to the solvent exposure [$y = 1.84x + 2.02$, where y is *o*-cresol ($\mu\text{mol/l}$) and x is the toluene concentration in the air ($\mu\text{mol/l}$)] (fig. 2). The practical lowest limit of detection of the toluene-related *o*-cresol was 2.5 $\mu\text{mol/l}$, which corresponded to a vapor concentration of 0.2 $\mu\text{mol/l}$ (5 ppm). This level seems to be caused by the minor urinary excretion of *o*-cresol also found for healthy control subjects (< 0.5 to 1.5 $\mu\text{mol/l}$). The excretion of *o*-cresol by control persons was confirmed by mass spectrometry (Hesso, unpublished results).

The removal of *o*-cresol after the exposure was rapid, the half-time varying from 3.5 to 4.6 h (fig. 3). The concentration of urinary *o*-cresol did not statistically (Student's *t*-test) differ from the non-exposed control concentrations 9 h after the end of the exposure. This situation prevailed also on the next morning. The highest *o*-cresol concentrations were detected 1 h after the end of the exposure. However, the excretion showed considerably greater variation than in samples taken immediately after the workshift. Therefore, the best correlation between the toluene vapor concentration in the air and the urinary *o*-cresol was seen in samples taken without the time lag.

Fig. 3. Excretion of *o*-cresol in the urine of the toluene-exposed persons during 8 h after the end of exposure. (The exposed printers were divided in two groups according to the toluene concentrations in the air, groups I and II.) The decay in the urinary *o*-cresol concentration is exponential, as shown by the fitted mathematical functions. Coefficient of correlation is given by r^2 .

DISCUSSION

The present findings indicate that the metabolic pathway involving the initial arene oxidation of toluene is active also in man. The rearrangement products are *p*- and *o*-cresol also in man. *p*-Cresol was also found in control urine. Its excretion may be caused by the intake of food, or it may result from microbial metabolism in the bowel (2). *o*-Cresol is suitable for the biological monitoring of toluene exposure, as a simple linear correlation exists, at least up to a toluene vapor concentration of 5 $\mu\text{mol/l}$ (120 ppm).

The rapid disappearance of urinary *o*-cresol after the end of exposure corresponds with the rapid oxidative elimination of absorbed toluene (5, 11, 12). The peak concentrations 1 h after the end of exposure might depend on the fact that the bladder acts as a reservoir allowing a considerable accumulation of constituents with rapid renal clearance. The greater variation in the excretion, as analyzed in these samples, may be caused by individual differences, as well as the characteristics of exposure, which cannot be estimated totally error-free when average concentrations are applied. A complicating factor may arise also from the fact that toluene enhances its oxidative removal in long-term exposure (6).

The measurement of the rearrangement products of arene oxidation may also have indirect toxicologic significance, as the arene oxides are highly reactive and may be bound to various nucleophilic sites in macromolecules (4, 9, 10). This finding contrasts with the side-chain oxidized products which are biologically less reactive.

In conclusion, the measurement of urinary *o*-cresol in toluene exposure is suitable for use as a biological exposure test. For this purpose, urine voided immediately after the end of exposure is to be recommended. The practical lowest limit of application lies at 0.2 $\mu\text{mol/l}$ (5 ppm), which corresponds to an *o*-cresol concentration of 2.5 $\mu\text{mol/l}$. Toluene exposure of 4 $\mu\text{mol/l}$ (100 ppm) corresponds to a urinary *o*-cresol concentration of 9.4 $\mu\text{mol/l}$ and 8 $\mu\text{mol/l}$ (200 ppm) to 16.8 $\mu\text{mol/l}$.

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