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Interoffice Communication

To Charles L. Whetstone, M.D.

From Oran D. Steffey

Date October 16, 1975

Subject RELATION OF BENZENE IN AIR TO LEVELS OF PHENOL DETECTED IN URINE

The following information will aid in answering your question as to the level of benzene in air exposure relative to the phenol level detected in the urine.

Docter and Zielhuis (1) suggested that "normal" values for urinary metabolites (phenol and phenol congeners) in individuals not exposed to benzene vary from 5-10 mg/liter with an upper limit of 15-20 mg/liter. Other estimates of the normal unexposed urinary phenol excretion are those of Diechmann and Schafer, (2) 11-42 mg, and Walkley et al, (3) an average of 30 mg/liter. Thus, urinary phenol levels in unexposed persons are well below the recommended biological level of 75 mg/liter.

Gas chromatographic methods have high specificity and provide for rapid determination of phenol in the urine. Detection of less than 0.1 ppm of benzene in air and 1 mg/liter of urine phenol is possible. The method of Sherwood and Carter (4) is the recommended method. This method is described in an attachment to this communication.

References:

(1) Docter, J.H., Zielhuis, R.L.: Phenol excretion as a measure of benzene exposure. Ann. Occup. Hyg. 10: 317-26, 1967.

(2) Diechmann, W., Schafer, L.J.: Phenol studies. Am. J. Clin. Path. 12: 129-43, 1942.

(3) Walkley, J.E., Pagnotto, L.D., Elkins, H.B.: The measurement of phenol in urine as an index of benzene exposure. Am. Ind. Hyg. Assoc. J. 22: 362-67, 1961.

(4) Sherwood, R.J., Carter, F.W.G.: The measurement of occupational exposure to benzene vapors. Ann. Occup. Hyg. 13: 125-46, 1970.

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IX. APPENDIX III

BIOLOGIC METHOD FOR SAMPLING AND ANALYSIS OF BENZENE

The recommended biologic method for urinalysis is derived from Sherwood and Carter. [102] It has been designed to determine the concentration of phenol and its conjugates, sulfate and glucuronide, in urine. It also determines orthocresol and meta- and paracresols. Urine is hydrolyzed with perchloric acid at 95 C, and the phenols and cresols are extracted with isopropyl ether and determined by gas chromatography.

Collection of Urine Samples

"Spot" urine specimens of about 100 ml are collected as close to the end of the working day as possible. If any worker's urine phenol level exceeds 75 mg/liter, procedures are instituted immediately to determine the cause of the elevated urine phenol levels and to reduce benzene exposure to the worker. Weekly specimens are collected as described above until 3 consecutive weekly determinations indicate that urinary phenol levels are below 75 mg/liter.

After thoroughly washing their hands with soap and water, workers shall collect urine samples from single voidings in clean, dry specimen containers having tight closures and at least a 120-ml capacity. Collection containers may be glass, waxcoated paper, or other disposable types if desired. Following collection of urine specimens, 1 ml of a 10% copper sulfate solution is added to each sample as a preservative, and samples are immediately stored under refrigeration, preferably at 0-4 C.

Refrigerated specimens will remain stable for approximately 90 days. If shipment of samples is necessary to perform analyses, the most rapid method available shall be employed utilizing acceptable packing procedures as specified by the carrier. Proper identification of each specimen shall include as a minimum, the worker's name, date, and time of collection.

Analytical

(a) Principle of the Method

Urine samples are treated with perchloric acid at 95 C to hydrolyze the phenol conjugates, phenyl sulfate, and phenyl glucuronide, formed as detoxification products following benzene absorption. The total phenol is extracted with diisopropyl ether and the phenol concentration is determined by gas chromatography analysis of the diisopropyl ether extract.

(b) Apparatus

(1) Gas chromatograph with a flame ionization detector and equipped with a 5-foot x 3/16-inch column packed with 2 w/w polyethylene glycol adipate on universal 'B' support. Operating conditions are as follows:

Column temperature	150 C
Detector temperature	200 C
Injection port temperature	200 C
Carrier gas	Nitrogen
Carrier gas flowrate	60 ml/min

(2) Water bath

(3) Glass-stoppered, 10-ml volumetric flasks

(4) 1-ml, 2-ml, and 5-ml volumetric pipets

(5) 5-μl syringe

(c) Reagents

(1) Phenol

(2) Perchloric acid

(3) Diisopropyl ether

(4) Distilled water

(d) Procedure

(1) Hydrolysis of Phenol Conjugates

Pipet 5 ml of urine into a 10-ml, glass-stoppered, volumetric flask. Add perchloric acid, mix by swirling, and transfer the lightly stoppered flask to a water bath at 95 C. After 2 hours, remove the flask from the water bath and allow to cool at room temperature.

(2) Diisopropyl ether extraction of phenol and cresols.

Pipet 1 ml of diisopropyl ether into the flask and adjust the volume to 10 ml with distilled water. Shake vigorously for 1 minute to extract the phenol and cresols. Allow the aqueous and ether layers to separate.

(3) Gas chromatographic analysis for phenol

Inject 5 μl of the diisopropyl ether layer into the gas chromatograph and record the attenuation and area of the phenol peak. Under the conditions described, phenol is eluted in 100 seconds, orthocresol in 130 seconds, and meta- and paracresols in 320 seconds.

(e) Standards Preparation

A 50 mg/liter standard aqueous solution of phenol is prepared. A 5-ml aliquot of the standard solution is then subjected to the hydrolysis, extraction, and gas chromatographic analysis procedures described under Procedure above.

(f) Calculations

Determine the phenol concentration in the urine by comparing the gas chromatographic peak area of the sample with that of the 50 mg/liter standard and adjust the value to a specific gravity of 1.024.

(g) Specific Gravity Correction

Due to the magnitude of correction which is required, samples having uncorrected specific gravities less than 1.010 shall be rejected and another sample shall be obtained.

Based on a survey of a large population in the United States in connection with urinary lead excretion, Levine and Fahy [139] found the mean specific gravity to be 1.024. Many investigators throughout the world now use this figure. Buchwald [130] in 1964 determined the mean specific gravity for residents in the United Kingdom to be 1.016, a value now frequently used for Northern Europeans. The importance of specific gravity adjustments can be seen in that a specific gravity of 1.016 will give results having two-thirds the value of those corrected to 1.024. It is important, therefore, that a value be chosen for standardization; since greater acceptance seems to be for 1.024, this value has been selected for adjustment of urinary concentrations of benzene recommended for biological monitoring.

$$\text{corrected concentration} = \frac{\text{observed concentration} \times 24}{\text{last 2 digits of sp gr (eg, 1.021)}}$$