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Determination of catechol and quinol in the urine of workers exposed to benzene

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ABSTRACT Time weighted average concentrations of benzene in breathing zone air (measured by diffusive sampling coupled with FID gas chromatography) and concentrations of catechol and quinol in the urine (collected at about 1500 in the second half of a working week and analysed by high performance liquid chromatography) were compared in 152 workers who were exposed to benzene (64 men, 88 women). The concentration of urinary metabolites was also determined in 131 non-exposed subjects (43 men, 88 women). There was a linear relation between the benzene concentrations in the breathing zone and the urinary concentrations of catechol and quinol (with or without correction for urine density) in both sexes. Neither catechol nor quinol concentration was able to separate those exposed to benzene at 10 ppm from those without exposure. The data indicated that when workers were exposed to benzene at 100 ppm about 25% of benzene absorbed was excreted into the urine as phenolic metabolites, of which 13·2%, 1·6%, and 10·2% are phenol, catechol, and quinol, respectively.

As early as 1953, Parke and Williams identified catechol and quinol, in addition to phenol, in the urine of rabbits given ^{14}C-benzene by mouth.[1] Regarding the biological monitoring by means of urine analysis, however, attention has been focused on phenol as an indicator of exposure to benzene.[2] Other phenolic urinary metabolites such as catechol and quinol are ignored, probably because of the technical difficulties in their analysis. A high performance liquid chromatographic (HPLC) method for the simultaneous determination of catechol and quinol[3 4] has been applied in our laboratory to the analysis of urine samples obtained from workers exposed to benzene, and the excretion of the two metabolites was related to the intensity of exposure to benzene. The results are presented in this report compared with previous findings[5] on excretion of phenol.

Materials and methods

EXAMINEES AND URINE COLLECTION

Urine samples previously analysed for phenol[5] were used. In brief, urine samples were collected from 152 workers (64 men, 88 women) exposed to benzene and 131 non-exposed workers (43 men, 88 women) at 1500 in the second half of a working week. At this time, concentrations of benzene metabolites are expected to reach the maximum in the urine of the workers exposed to benzene throughout the week.[6]

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BENZENE CONCENTRATION IN BREATHING ZONE AIR

The time weighted (seven hour) average exposure measured by diffusive sampling[7] is cited from a previous publication.[5] It was assumed that benzene exposure follows a log normal distribution[8 9] and distribution is therefore expressed in terms of geometric mean (GM) and geometric standard deviation (GSD).

HPLC ANALYSIS OF URINE FOR CATECHOL AND QUINOL

Each urine sample was heated at 100°C for one hour in the presence of hydrochloric acid (final concentration, 1·75%) for hydrolysis. The hydrolysate, 2·5 ml, was added to 0·2 ml of 2 mg 3,5-xylenol/ml methanol (as an internal standard) and extracted with 3 ml carbon disulphide-diethyl ether (1:1 by volume) by vigorous shaking for five minutes. After centrifugation at −10°C for 10 minutes for separation, the lower

organic layer was taken, desiccated with 0·5 g sodium sulphate anhydrate, and evaporated at 25°C under a nitrogen stream to dryness. The residue was taken up in 0·5 ml acetonitrile-water (3:7 by volume), a portion (5 to 10 μl per injection) of which was applied to HPLC analysis.[3,4] The HPLC used was a Hitachi Model 635 equipped with a Hitachi No 3056 column (3 mm in inner diameter and 150 mm in length, kept at 45°C) and an autosampler (Model KSST-60, Kyowa-Seimitsu Co, Tokyo, Japan). The mobile phase was a mixture of acetonitrile-acetic acid-water (15·0%:1·5%:83·5%, by volume) and was allowed to flow at a rate of 1·5 ml/min. The metabolites were detected spectrophotometrically at 280 nm. The detection limit was 0·5 mg/l for catechol and 1·0 mg/l for quinol. In preliminary studies with urine samples rich in catechol and quinol conjugates it was found that the hydrolysis conditions used were optimal and that no benzoquinone (an oxidation product of quinol[10]) or 1,2,4-benzenetriol was detected on the chromatogram when urine samples from workers exposed to benzene at high concentrations were analysed. The metabolite concentrations were expressed either with or without correction for creatinine concentration[11] or for specific gravity of 1·016.[12] For the evaluation on a group basis, a log normal distribution was assumed. The creatinine concentration was measured colorimetrically[13] and the specific gravity by refractometry.

When authentic catechol (at five levels up to 1000 mg/l) and quinol (at five levels up to 500 mg/l) were dissolved either in water or in 10 control urine samples (of low catechol or quinol contents), urine and water samples gave the same calibration lines; the former values were 97·1% (with a coefficient of variation of 4·6%) of the latter for catechol and 101·7% (with a coefficient of variation of 3·5%) for quinol. To examine the reproducibility of the analyses, a urine sample from an exposed subject (containing about 50 mg catechol and about 250 mg quinol/l) was divided into eight portions and analysed simultaneously. The coefficient of variation was 3·6% for both catechol and quinol analyses.

Results and discussion

CONCENTRATIONS OF CATECHOL AND QUINOL IN URINE OF THE NON-EXPOSED SUBJECTS

Both the distribution of catechol and quinol concentrations in the urine samples obtained from non-exposed subjects showed positive skewness. The highest frequency of catechol values was in the range of 7–9 mg/l and that of quinol concentrations in the range of 1–3 mg/l (fig 1). The mean, mode, and median were determined before and after logarithmic transformation to examine the fitness with normal and log normal distribution. For example, the calculation with the observed catechol values of 88 non-exposed women gave 13·3, 7·5, and 12 mg/l as the mean, mode, and median before transformation, and the logarithmic values were 1·04, 0·99, and 1·08 respectively. In the case of quinol the pretransformation values were 7·6, 1·9, and 5 mg/l and the logarithmic values 0·62, 0·64, and 0·70. Thus a log normal distribution was assumed for both phenolic compounds for statistical evaluation. The GMs (GSDs in parentheses) of catechol and quinol concentrations in the urine of the non-exposed subjects are summarised in table 1. The concentrations are given as observed values and the values corrected for creatinine concentrations and for specific gravity. Whereas the GSD was generally less than 2·0 in the case of catechol, a GSD of larger than 4·0 was often observed with quinol, indicating a wider variation in the latter than in the former. There was no significant ($p > 0.05$) sex difference in the levels of the two compounds.

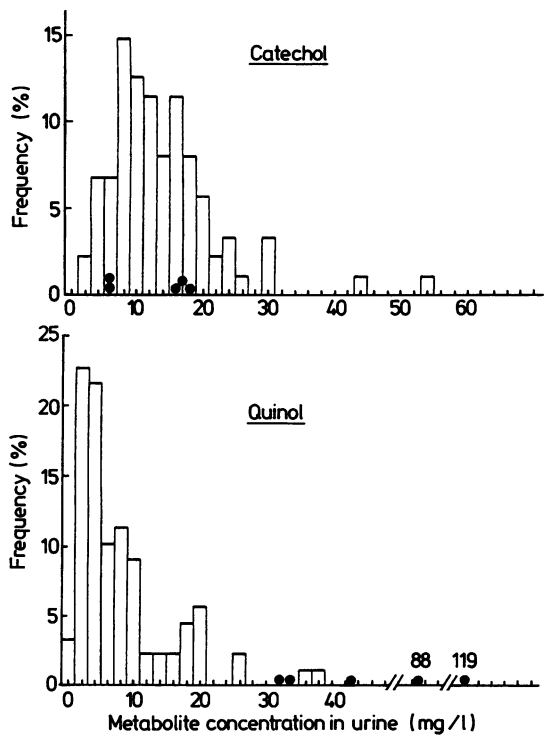

Fig 1 *Distribution of catechol and quinol in urine of 88 non-exposed women. Observed values are shown. Catechol and quinol values in urine of five women exposed to benzene at 9–14 ppm are spotted in histograms to show difficulty of separating those exposed to benzene at about 10 ppm from those not exposed.*

Table 1 *Catechol and quinol concentrations in urine samples from non-exposed subjects*

Sex	No of subjects	Observed value* (mg/l)	Value* corrected for: Creatinine (mg/g)	Value* corrected for: Specific gravity (mg/l)
		Catechol		
Men + women	131	10·63 (1·92)	13·32 (1·67)	10·88 (1·68)
Men	43	9·77 (2·01)	13·03 (1·71)	11·42 (1·65)
Women	88	11·08 (1·87)	13·47 (1·65)	10·63 (1·69)
		Quinol		
Men + women	131	4·19 (4·66)	5·43 (3·90)	4·30 (4·17)
Men	43	4·24 (5·42)	6·63 (2·68)	5·85 (2·61)
Women	88	4·17 (4·35)	4·93 (4·51)	3·70 (4·91)

There is no statistically significant ($p > 0.05$) difference between the two sexes.
*Geometric mean (geometric standard deviation).

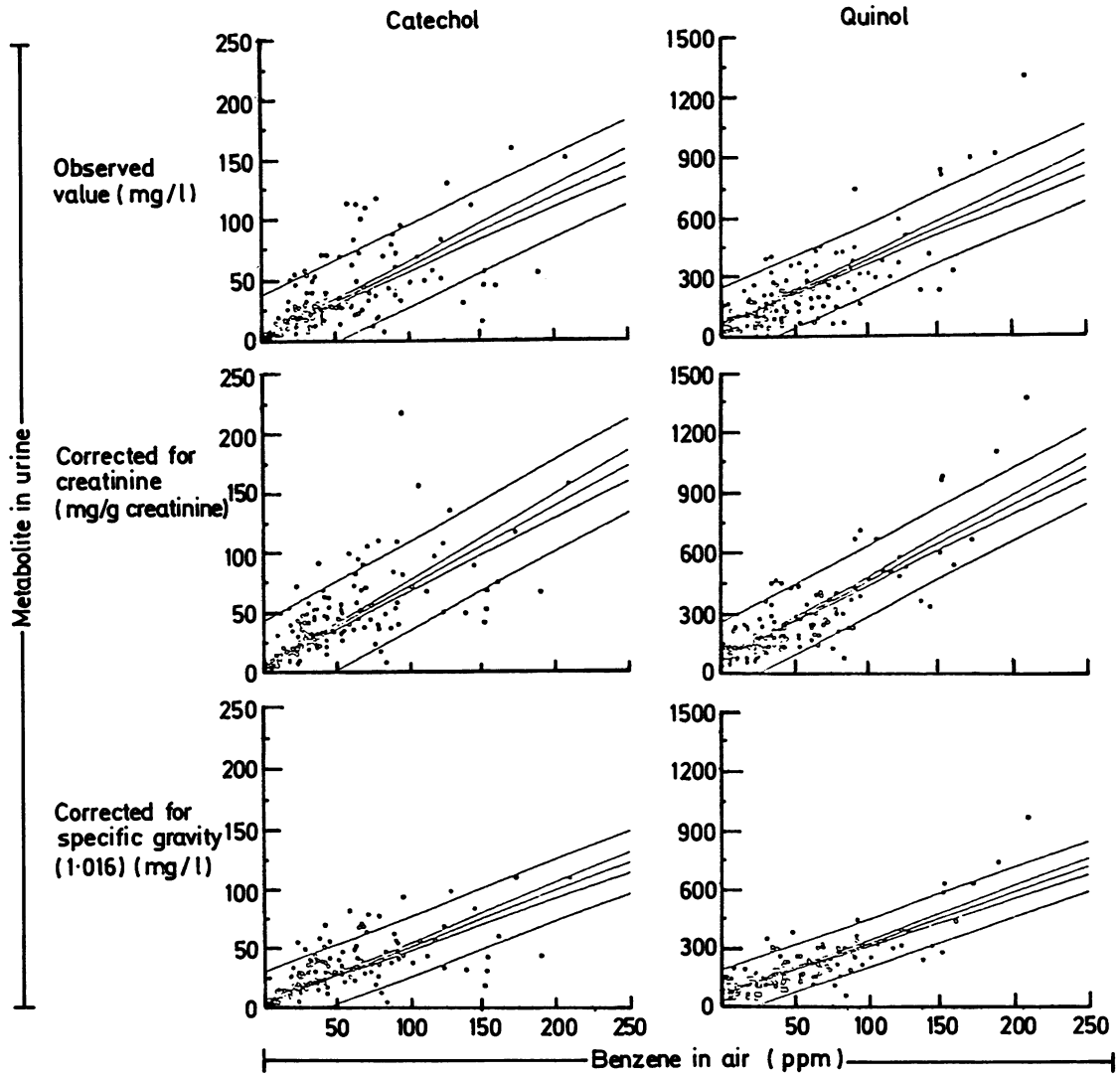

Fig 2 *Relation between benzene in breathing zone air and metabolites (catechol and quinol) in urine. Points indicate individual values. Data from men and women are presented in combination. Lines and curves are calculated regression line (line in centre), 95% confidence ranges of sample means (curves close to regression line), and 95% confidence ranges of individual samples (outmost lines).*

LINEAR INCREASE OF CATECHOL AND QUINOL CONCENTRATIONS IN URINE AS A FUNCTION OF BENZENE CONCENTRATION IN BREATHING ZONE AIR

The concentrations of catechol and quinol in the urine of the exposed workers are tabulated by sex and by workshop in table 2, in which the workshops are arranged in the increasing order of benzene concentration. Although the number of workers examined was small in some workshops, both metabolite concentrations increased with increasing benzene exposure. The quinol concentrations were several times higher than the catechol concentrations. The high quinol/catechol ratio resembles the results of an in vivo study in which rabbits were fed ^{14}C-phenol[14] and an in vitro assay in which ^{14}C-phenol was incubated with rat liver microsomes[15] (with the quinol/catechol ratio = 10–20 in both cases) rather than that of an in vivo ^{14}C-benzene feeding study with rabbits (the quinol/catechol ratio = 2).[1] Comparison with phenol concen-

Table 2 *Concentrations of catechol and quinol in urine of workers exposed to benzene at various workshops*

			*Catechol concentration**			*Quinol concentration**		
				Value corrected for			*Value corrected for*	
Workshop	*No of workers*	*Benzene in breathing zone air (ppm)*	*Observed value (mg/l)*	*Creatinine (mg/g)*	*Specific gravity (mg/l)*	*Observed value (mg/l)*	*Creatinine (mg/g)*	*Specific gravity (mg/l)*
Men								
A	3	1·0 (1·00)	11 (2·24)	12 (1·92)	13 (1·86)	10 (2·39)	11 (2·04)	12 (2·17)
B	24	11·1 (2·40)	12 (1·61)	14 (1·54)	12 (1·61)	39 (2·21)	48 (2·37)	39 (2·58)
C	20	32·6 (1·42)	19 (1·88)	26 (1·38)	21 (1·41)	134 (1·62)	185 (1·44)	150 (1·45)
D	2†	7, 76	11, 22	20, 21	18, 22	37, 77	33, 146	37, 124
E	15	60·2 (1·28)	38 (1·74)	43 (1·37)	34 (1·50)	257 (1·73)	290 (1·41)	229 (1·40)
Women								
A	1†	1	10	21	15	12	25	18
B	19	18·1 (2·79)	22 (2·73)	31 (2·15)	24 (2·01)	100 (2·91)	138 (2·42)	104 (2·18)
C	37	42·4 (2·49)	35 (1·85)	43 (1·96)	33 (1·78)	201 (2·15)	246 (2·12)	186 (1·93)
D	2†	5, 37	15, 16	22, 24	15, 17	3, 77	5, 128	3, 88
E	29	76·4 (1·58)	39 (2·01)	53 (1·70)	40 (1·62)	277 (2·02)	371 (1·74)	278 (1·68)

*Geometric mean (geometric standard deviation).
†Individual values are shown.

Table 3 *Correlation between breathing zone levels of benzene and urinary concentrations of catechol and quinol*

		Catechol			*Quinol*		
Measurement group	*No of subjects**	*A*†	*B*†	*r*‡	*A*†	*B*†	*r*‡
Observed value (mg/l):							
Men + women	283	0·537	10·2	0·808	3·395	24·3	0·793
Men§	108	0·454	9·8	0·663	3·788	12·3	0·782
Women‖	175	0·546	11·2	0·831	3·308	29·6	0·792
Women¶	159	0·419	13·4	0·710	4·474	14·7	0·807
Value corrected for creatinine (mg/g):							
Men + women	283	0·636	12·8	0·858	4·025	33·6	0·811
Men§	108	0·411	13·7	0·745	4·049	24·3	0·794
Women‖	175	0·667	14·0	0·877	3·992	40·0	0·811
Women¶	159	0·569	15·6	0·763	5·774	15·1	0·843
Value corrected for specific gravity (mg/l):							
Men + women	283	0·426	11·3	0·851	2·839	29·5	0·821
Men§	108	0·326	11·9	0·674	3·245	21·8	0·779
Women‖	175	0·440	11·7	0·879	2·764	31·6	0·831
Women¶	159	0·391	12·6	0·774	4·131	13·8	0·891

*Including non-exposed subjects (43 men, 88 women).
†Slope (A) and the intercept on the Y axis (B) in the equation as Y = AX + B, where Y is the urinary concentration of catechol or quinol (unit; as described in the table) and X is the breathing zone concentration of benzene (ppm).
‡p for correlation coefficient (r) is $<0·01$ for all groups.
§Exposed up to 92 ppm.
‖Exposed up to 210 ppm.
¶Women exposed to less than 100 ppm.

Table 4 *Comparison of the lower 95% confidence limit of metabolite concentration in the urine of workers exposed to 10 ppm benzene with the upper 95% confidence limit of metabolite concentration of non-exposed subjects (number in parentheses)*

Sex	Observed value (mg/l)	Value corrected for: Creatinine (mg/g)	Value corrected for: Specific gravity (mg/l)
		Catechol	
Men + women	13·7 (39·2)	17·3 (37·1)	14·4 (30·7)
Men	11·7 (38·7)	15·8 (38·1)	13·3 (31·1)
Women	13·9 (39·5)	18·1 (36·7)	14·2 (30·4)
		Quinol	
Men + women	45·6 (91·0)	59·4 (82·6)	48·3 (74·8)
Men	35·6 (124·6)	48·9 (47·6)	41·1 (39·9)
Women	43·9 (78·9)	58·3 (100·3)	46·0 (89·2)

trations published in a preceding paper showed that the quinol concentrations were almost as high as that of phenol for all levels of benzene exposure. This observation in man differs from the findings of the benzene feeding study with rabbits in which the amount of phenol detected in urine was about five times higher than that of quinol.[1]

The relation between the benzene concentration in breathing zone air and the two metabolite levels in the urine was examined by pooling the data from the exposed and non-exposed subjects. The results are shown in fig 2; the data from men and women are shown in combination, with or without the correction for creatinine concentration and specific gravity. Although the scattering was rather wide in some cases—for example, quinol in female urine after correction for creatinine—there were significant ($p < 0·01$) linear correlations between exposure and the excretion of metabolites as shown in table 3. As some female workers were exposed to benzene up to 210 ppm whereas the maximum concentration for men was 92 ppm, women exposed to less than 100 ppm benzene were selected for the comparison of excretion between the two sexes. This indicated no sex difference in the biotransformation of benzene to these two metabolites.

Table 4 shows that the exposure to benzene at 10 ppm gave urinary excretion values that were overlapped by those of non-exposed subjects. The overlap is illustrated by the superimposition of urinary catechol and quinol concentrations of five women exposed to 9–14 ppm benzene on the histograms of 88 non-exposed women (fig 1).

As data on three major phenolic metabolites are made available by the combination of the present observations on catechol and quinol with the previous one on phenol,[5] it is possible to make a quantitative cross sectional estimation of input-output balance on benzene metabolism in workers exposed to benzene—for instance, at 100 ppm level. Assuming as in the previous paper that about 50% of benzene inhaled will be absorbed through the lungs, and that the rates of respiration and urine excretion will be 15 l/min and 1 ml/min, respectively, the amount of benzene absorbed at 100 ppm is 2392·5 μg/min. The amount of benzene excreted in urine as catechol (quinol is in parentheses) is

53·7 (339·5) mg/l × 1 × 10^{-3} l/min = 53·7 (339·5) μg/min or 38·1 (240·8) μg/min as benzene [= 53·7 (339·5) μg/min × 78·11/110·00] where 78·11 and 110·11 are the molecular weights of benzene and catechol or quinol, respectively. The calculated excretion of phenol is 316·7 μg/min.[5] Thus 596 μg/min or about 25% of benzene absorbed is excreted in the form of these three urinary metabolites, of which 13%, 2%, and 10% are as phenol, catechol, and quinol, respectively.

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