Urinary excretion of phenol, catechol, hydroquinone, and muconic acid by workers occupationally exposed to benzene

N Rothman, W E Bechtold, S-N Yin, M Dosemeci, G-L Li, Y-Z Wang, W C Griffith, M T Smith, R B Hayes

Occupational Studies Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, EPN 418, Bethesda, MD, USA
N Rothman
M Dosemeci
R B Hayes

Inhalation Toxicology Research Institute, PO Box 5890, Albuquerque, NM, USA
W E Bechtold
W C Griffith

Chinese Academy of Preventive Medicine, Institute of Occupational Medicine, 29 Nan Wei Road, Beijing, China
S-N Yin
G-L Li

Shanghai Hygiene and Anti-Epidemic Center, Department of Occupational Health, 1380 Zhong Shan Road West, Shanghai, China
Y-Z Wang

School of Public Health, University of California, Warren Hall, Berkeley, CA, USA
M T Smith

Correspondence to:
Dr Nathaniel Rothman, NIH/NCI EPN 418, Bethesda, MD 20892, USA. Telephone 001 301 496 9093; email: rothmann@epndce.nci.nih.gov

Accepted 25 February 1998

Abstract
Objectives—Animal inhalation studies and theoretical models suggest that the pattern of formation of benzene metabolites changes as exposure to benzene increases. To determine if this occurs in humans, benzene metabolites in urine samples collected as part of a cross sectional study of occupationally exposed workers in Shanghai, China were measured.
Methods—With organic vapour monitoring badges, 38 subjects were monitored during their full workshift for inhalation exposure to benzene. The benzene urinary metabolites phenol, catechol, hydroquinone, and muconic acid were measured with an isotope dilution gas chromatography mass spectroscopy assay and strongly correlated with concentrations of benzene air. For the subgroup of workers (n=27) with urinary phenol >50 ng/g creatinine (above which phenol is considered to be a specific indicator of exposure to benzene), concentrations of each of the four metabolites were calculated as a ratio of the sum of the concentrations of all four metabolites (total metabolites) and were compared in workers exposed to >25 ppm $v \leqslant 25$ ppm.
Results—The median, 8 hour time weighted average exposure to benzene was 25 ppm. Relative to the lower exposed workers, the ratio of phenol and catechol to total metabolites increased by 6.0% (p=0.04) and 22.2% (p=0.007), respectively, in the more highly exposed workers. By contrast, the ratio of hydroquinone and muconic acid to total metabolites decreased by 18.8% (p=0.04) and 26.7% (p=0.006), respectively. Similar patterns were found when metabolite ratios were analysed as a function of internal benzene dose (defined as total urinary benzene metabolites), although catechol showed a more complex, quadratic relation with increasing dose.
Conclusions—These results, which are consistent with previous animal studies, show that the relative production of benzene metabolites is a function of exposure level. If the toxic benzene metabolites are assumed to be derived from hydroquinone, ring opened products, or both, these results suggests that the risk for adverse health outcomes due to exposure to benzene may have a supralinear relation with external dose, and that linear extrapolation of the toxic effects of benzene in highly exposed workers to lower levels of exposure may underestimate risk.
(*Occup Environ Med* 1998;55:705–711)

Occupational exposure to benzene has been causally associated with increased risk of aplastic anaemia, myelodysplastic syndromes, and acute non-lymphocytic leukaemia.[1–3] The evaluation of risk posed by exposure to relatively low concentrations of benzene from occupational and environmental sources is an active area of research, given the ubiquity of benzene in the general environment.[1] Because human data are sparse on risk of leukaemia due to low concentrations of benzene, the risk assessment process has generally relied on the extrapolation of observations made in highly exposed populations.[4] However, extensive evaluation of the effects of dose, dose rate, route of exposure, and species on benzene absorption, metabolism, and excretion in experimental animals[5–8] has shown that the relative proportions of benzene metabolites are highly dependent on dose level, raising concerns about high to low dose extrapolations.[7] For example, a model developed by Schlosser *et al*[9] based on benzene and phenol metabolism in mouse and rat liver microsomes suggests that as benzene concentration increases, metabolism of phenol to hydroquinone, a more toxic benzene metabolite along with its triol and quinone derivatives,[10–13] should decrease due to competition for *P*-450 activation.

By contrast with the extensive database on benzene metabolism in animals, relatively little is known about potential benzene metabolite shifts in humans over wide ranges of exposure to benzene.[14] To gain insight into the early biological effects of exposure to benzene in currently exposed workers, we carried out a cross sectional study of workers exposed to benzene and unexposed controls in Shanghai, China.[15] We have previously shown a dose-response relation between exposure to benzene and haematotoxic, aneuploidigenic, clastogenic, and genotoxic events in this population.[15–19] Here, we report how the patterns of the four most important urinary metabolites of benzene—phenol, hydroquinone, catechol, and muconic acid vary over a broad range of exposure.

Materials and methods

SUBJECT ENROLMENT

Details of the study design and demographic characteristics of study subjects have been previously reported.[15] Briefly, three factories in China were selected in which workers were occupationally exposed to benzene with minimal coexposures to other aromatic solvents. Controls were selected from workplaces without exposure to benzene and were frequency matched to the exposed subjects on age and sex. Forty four workers exposed to benzene and 44 controls were enrolled into the study. Exclusion criteria for subjects were a history of cancer, therapeutic radiation, chemotherapy, or current pregnancy. Informed consent was obtained from all study participants with Institutional Review Board approved procedures. Data were collected by interview on demographics and on current tobacco and alcohol use, and the height and weight of each subject were measured.

EXPOSURE ASSESSMENT

Occupational exposure to benzene and other organic vapours was monitored with passive dosimeters (3M No 3500, St Paul, MN) worn by each worker for a full work shift on the day of the study. Badges were analysed by the badgemakers' methods, which used gas chromatography (GC) with flame ionisation detection for benzene, toluene, and xylene. For quality control, two separate badges worn by 10 workers were analysed in two laboratories. Analytical results from these duplicate measurements were highly correlated (Spearman, $r=0.98$, $p<0.0001$).[15]

The workers exposed to benzene were monitored for personal exposure to benzene in air for 5 days. Workers in factory 1 worked about 5–6 hour work shifts; for almost all workers, most of the exposure to benzene occurred during a 2.5–3 hour period. A spot urine sample was collected at the end of this high exposure period. Workers in factories 2 and 3 worked 8 hour workshifts, and a spot urine sample was collected at the end of the work shift. Urine samples were immediately frozen on dry ice and stored for later shipment.

In factory 1, most workers used half face respirators fitted with activated charcoal cartridges that were changed when the workers could detect the odour of benzene (about every other day). The fit of the respirators was not tested. Respirators were not used in either factory 2 or 3.

Control subjects were enrolled from two workplaces. In the first workplace, which manufactured sewing machines, most subjects were monitored for exposure to benzene with passive dosimeters for one day on any of six different sampling days. Workers in the second control workplace, which was administrative, were not monitored. All control subjects provided a spot urine sample at a clinic.

MATERIALS

The reagents phenol, hydroquinone, catechol, and muconic acid (Aldrich Chemical, Milwaukee, WI, USA), ethyl acetate (Burdick and Jackson, Muskegon, MI, USA), sodium sulphate (Fisher Scientific, Pittsburgh, PA, USA), and N,O-bis (trimethylsilyl) trifluoracetamide (BSTFA) (Pierce Manufacturing, Appleton, WI, USA) used in this study were of the highest purities available. [$^{13}C_6$] Benzene metabolite standards were biosynthesised in rats as previously described,[20] except that [$^{13}C_6$] benzene was used instead of [H_6] benzene. In previous studies with deuterated metabolite standards, all samples containing internal standards were kept in liquid nitrogen until needed to avoid exchange of the labile deuterium.[20] Because of the stability of the ring labelled carbon atoms, urine samples in this study were conveniently stored at -80°C until needed. Concentrations of the [$^{13}C_6$] benzene metabolites used as internal standards were measured against a primary standard solution.

ANALYSIS OF URINE FOR BENZENE METABOLITES

Samples of urine were analysed blinded with respect to exposure. They were thawed at room temperature, and 200 µl distilled water was added to vials containing 200 µl urine. Internal standard solution (100 µl) was added, and the contents were mixed with 100 µl concentrated HCl. The vials were capped, incubated at 100°C for 60 minutes, and allowed to cool. The solutions were extracted with 2 ml ethyl acetate. The ethyl acetate layer was removed and dried with a small scoop of sodium sulphate. The sample was decanted, and the drying agent was rinsed with 1 ml ethyl acetate. The ethyl acetate aliquots were combined and
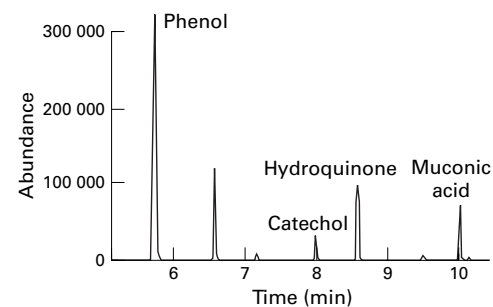

Figure 1   Selected ion chromatogram for the benzene metabolites phenol, catechol, hydroquinone, and muconic acid. The sample of urine was acquired from a worker exposed to >31 ppm time weighted average over an 8 hour day. The ions measured are presented in the text. All six ions were simultaneously collected throughout the chromatogram.
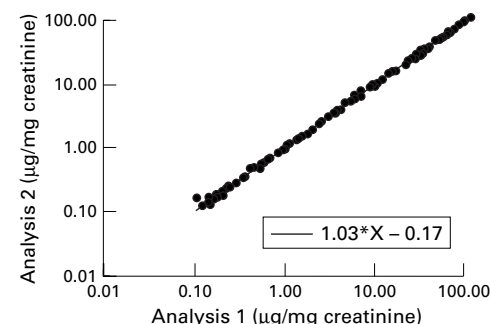

Figure 2   Duplicate measurement of muconic acid in worker urine samples. The units for the axis are µg/mg creatinine ($R^2=0.999$).

Table 1　Correlation between benzene exposure category and urinary phenol, catechol, hydroquinone, and muconic acid in workers occupationally exposed to benzene and controls studied in Shanghai, China, 1992

Group	Benzene 8h TWA (ppm)	Phenol (ng/g creatine)	Catechol (ng/g creatinine)	Hydroquinone (ng/g creatinine)	Muconic acid (ng/g creatinine)
Control (n=17):					
Median	ND	17.3	3.2	1.6	0.2
Range		3.7–55.3	1.6–8.7	0.9–6.7	0.1–0.8
25–75 Percentile		8.7–27.0	2.8–3.7*	1.3–3.0	0.2–0.2
Exposed (1–≤25 ppm, n=20):					
Median	9.5	55.6	7.2	16.4	14.6
Range	0.8–25.1	16.5–487.2	2.5–84.2	3.8–82.2	2.6–63.4
25–75 Percentile	4.7–25.1	32.7–102.0	5.2–14.6†	9.8–31.9	6.4–28.1
Exposed (>25ppm, n=18):					
Median	74.0	350.6	67.3	65.3	49.0
Range	33.1–331.7	27.7–516.7	7.0–81.4	8.3–196.6	5.3–103.4
25–75 Percentile	51.4–168.1	246.3–397.1	50.8–74.4	39.7–83.9	37.0–73.1
Spearman R		0.82	0.82	0.86	0.86
p		<0.0001	<0.0001	<0.0001	<0.0001

*n=16.
†n=19.

evaporated to 200 µl under nitrogen. The analyses were derivatised by adding 100 µl BSTFA and incubated at 60°C for 30 minutes.

A Hewlett Packard 5890/5970B gas chromatograph mass selective (GC-MS) detector was used for all analyses. This was equipped with a Restek RT$_x$-5 fused silica capillary column, 25 m (length) × 0.23 mm (internal diameter) with a 0.25 µm film thickness. The initial column temperature was 80°C for 1 minute, followed by a temperature gradient of 12°C/min to 265°C. All injections (1 µl) were split less with the splitter on at 30 s. Ions 166, 254, 254, and 271 (M+, M+, M+, and (M - CH$_3$)+, respectively) were monitored for phenol, catechol, hydroquinone, and muconic acid, respectively, while ions 172, 260, 260, and 277 were monitored for the isotopically labelled metabolites.

Standard curves were made by adding identical aliquots of internal standard solution to varying concentrations of analysed solutions in methanol (0.25-5.0 µg). The resulting mixtures were blown to dryness under a stream of dry nitrogen and were derivatised as described above. After analysis by gas chromatography mass spectroscopy (GC-MS), the absolute amounts of metabolite standard versus the ratio of integrated peak areas for analysed ions relative to respective internal standard ions were plotted. Standard curves were consistently linear with R^2 >0.99 for all metabolites. This same ratio was calculated for samples and amounts of metabolites computed from the standard curve. Urinary creatinine was measured by the Instrumentation Laboratories test creatinine with a Monarch Instrument (Amherst, NH) 1000 centrifugal chemical analyser.

The benzene metabolites were readily separated by GC-MS, with little interference (fig 1). Samples of urine were diluted with deionised water because of the high concentrations of metabolites. Therefore, method detection limits were not computed, as all metabolites were easily measurable in all urine samples processed. Method reproducibility was assessed by measuring all urine samples in duplicate and calculating the means of the percentage differences. Results for the duplicate analysis of muconic acid are shown in fig 2. The means of the percentage differences of the measurements for phenol, catechol, hydroquinone, and muconic acid were 3.0, 9.0, 6.4, and 5.3 respectively.

STATISTICAL ANALYSIS

Thirty eight of the 44 exposed workers provided a urine sample on the same day that the air samples were collected. Samples from a subsample (n=17) of the 44 controls, frequency matched to exposed workers by age, sex, and current smoking status, were analysed. Summary data for benzene in air and urinary metabolite concentrations in these subjects are presented as median, highest and lowest value, and interquartile range (25th and 75th percentile of data) because the data were highly skewed. The relation between exposure category and benzene concentrations in air and metabolite concentrations was tested by the Spearman rank order correlation test. One data point for urinary hydroquinone was twice as high as the next greatest value and a formal outlier (>1.5 times the interquartile range). Regression diagnostics, described below, showed that it was also a high influence point. Therefore, data from this subject were deleted from subsequent analyses.

Among the subgroup of workers with urinary phenol >50 ng/g creatinine (n=27), concentrations of benzene in air and urinary

Table 2　Ratio of each benzene metabolite to total benzene metabolites by exposure level in workers exposed to benzene with >50 ng/g creatinine urinary phenol

Group	Benzene 8h TWA (ppm)			Phenol	Catechol	Hydroquinone	Muconic acid
	Median	Range	25–75 Percentile	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
1–≤25 ppm (n=11)*	20.5	4.8–25.1	11.7–21.7	0.61 (0.04)	0.09 (0.02)	0.16 (0.03)	0.15 (0.03)
>25 ppm (n=16)†	78.0	40.3–331.7	57.4–178.2	0.65 (0.05)	0.11 (0.02)	0.13 (0.03)	0.11 (0.03)
p Value				0.04	0.007	0.04	0.006

*Nine subjects with <50 ng/g creatinine urinary phenol deleted.
†One subject with <50 ng/g creatinine urinary phenol and one subject with an outlying value of hydroquinone deleted.
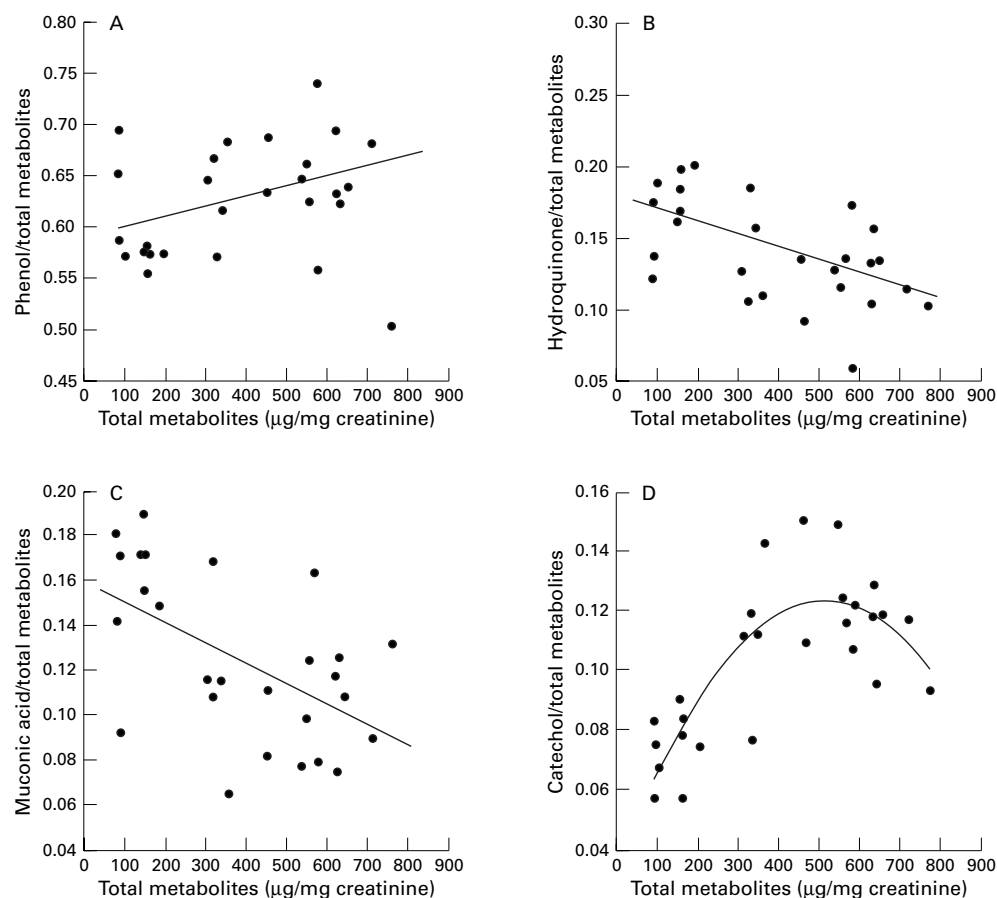
Figure 3   Relation between the concentrations of total metabolites in urine and the ratios of phenol, hydroquinone, muconic acid, and catechol to total metabolites. (A) Phenol/total metabolites=0.0001 (total metabolites)+0.59. R^2=0.21; model p=0.02. (B) Hydroquinone/total metabolites=−0.000089 (total metabolites)+0.18; R^2=0.31; model p=0.002. (C) Muconic acid/total metabolites=−0.000093 (total metabolites)+0.16; R^2=0.31; model p=0.003. (D) Catechol/total metabolites=0.00035 (total metabolites)−0.00000035 (total metabolites^2)+0.035; R^2=0.67; model p< 0.0001.

metabolites in workers exposed to ≤25 ppm were compared with concentrations in workers exposed to >25 ppm by the *t* test. Subjects with phenol concentrations <50 ng/g were excluded, as phenol is not specific for exposure to benzene at lower concentrations.[21]

The relation between total urinary metabolites (used to estimate internal benzene dose) and the ratio of each metabolite to total metabolites was evaluated by multiple linear regression. Both linear and quadratic relations between total metabolites and each ratio were tested, and potential confounding by age, sex, current alcohol consumption, tobacco use, and body mass index (a measure of obesity calculated as weight (kg)/ height (m^2) was evaluated. Confounding was considered to be present if the regression coefficients for total urinary metabolites changed by >15% in either direction. The appropriateness of final models was evaluated by analysis of residuals and tests to identify high influence values. Two sided tests were calculated throughout; p values <0.05 were considered adequate to reject the null hypothesis. Statistical analyses were performed with SAS for personal computer version 6.04.[22]

Results

Summary measures of urinary concentrations of metabolites of benzene are presented in table

1 for controls, workers exposed to ≤25 ppm benzene, and workers exposed to >25 ppm benzene, and were highly correlated with exposure category (Spearman *R*>0.80, p<0.0001, for all metabolites). The proportion of total metabolites made up by each specific metabolite is presented for workers exposed to ≤25 ppm and workers exposed to >25 ppm in table 2. Comparisons of the percentage change occurring in this proportion were made between the lower and higher exposed groups; phenol increased by 6% (p=0.04), catechol increased by 22% (p=0.007), hydroquinone decreased by 19% (p=0.04), and muconic acid decreased by 27% (p=0.006).

Individual concentrations of benzene in air, as continuous measures, could not be confidently used to provide more detailed insight into the nature of the relation between dose of benzene and metabolite ratio because exposure to benzene received by workers who wore respirators could only be approximated by the air monitoring data. Therefore, we used the sum of the concentrations of the major metabolites as a surrogate measure of air exposure. Studies in rats and mice have shown that these metabolites, as their conjugates, represent the overwhelming majority of the metabolites found in urine.[5 6] Scatter plots are presented for
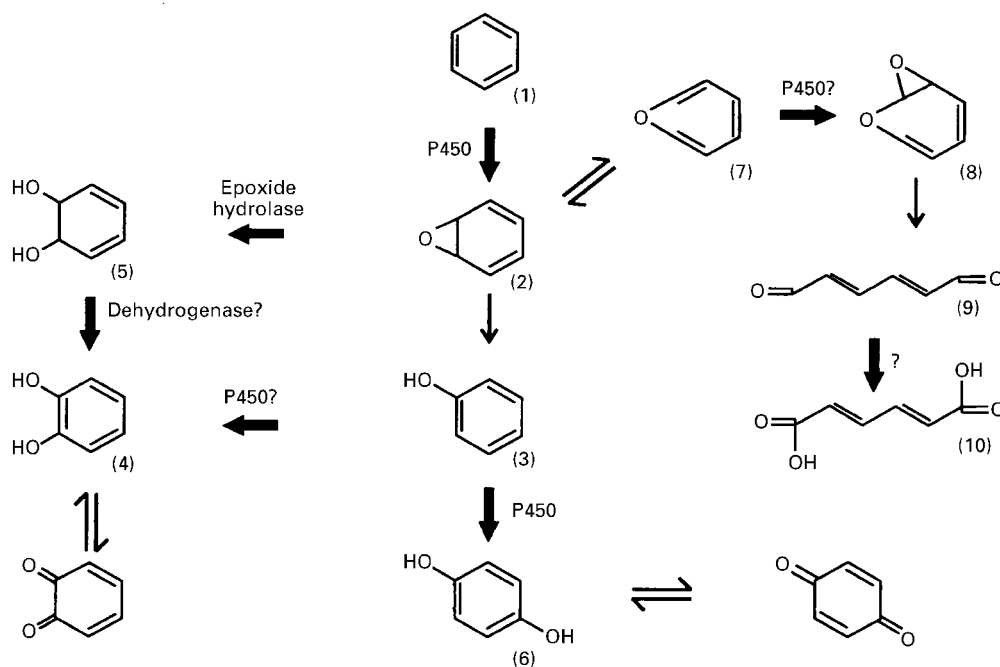
Figure 4   Metabolic scheme for benzene; (1) benzene; (2) benzene oxide; (3) phenol; (4) catechol; (5) trans-1 dehydroxy-1,2-dehydrobenzene; (6) hydroquinone; (7) oxepin; (8) epoxyoxepin; (9) muconaldehyde; (10) muconic acid.

each metabolite ratio as a function of internal dose in figure 3 A–D.

As total metabolites increased, the proportion made up by phenol increased (p=0.02, fig 3 A) whereas the proportion made up by hydroquinone and muconic acid decreased (p=0.002 and 0.003, respectively, fig 3 B, C), similar to the change in metabolite patterns found as a function of air concentration shown in table 2. The shift in the proportion of total metabolites made up by catechol showed a more complex, quadratic relation, initially increasing and then falling (fig 3 D). Multiple linear regression analysis showed that there was no confounding of these relations by age, sex, body mass index, current alcohol consumption, or current tobacco use.

As noted previously, samples of urine were collected at the end of the high exposure period in factory 1 and at the end of a full 8 hour work shift in factories 2 and 3. To determine if the difference in collection times influenced the results, we stratified the analysis of urinary metabolite ratios among lower and higher exposed workers presented in table 2 by factory (factory 1 *v* factories 2 and 3, combined). The direction of the change in metabolite proportions from lower to higher exposed workers was the same in each group (phenol 2% increase in factory 1 and 12% increase in factories 2 and 3; catechol 26% and 29% increase, respectively; hydroquinone 12% and 22% decrease, respectively; muconic acid 17% and 32% decrease, respectively). Also, a factory variable (factory 1 *v* factories 2 and 3) was not significant in the regression analysis of any metabolite outcome and did not confound the associations presented in figure 3 A–D.

Discussion

We analysed urinary benzene metabolites in a cross sectional study of workers exposed to a wide range of benzene concentrations and unexposed controls. Compared with workers exposed to ⩽25 ppm, the relative amounts of muconic acid and hydroquinone decreased among more highly exposed workers, whereas the relative amounts of catechol increased. The relative amounts of phenol increased as well, but to a smaller extent (6%). Linear regression analysis of these outcomes with total metabolites as a measure of internal dose showed that these relations were linear for muconic acid, hydroquinone, and phenol, and quadratic for catechol.

Exposure to benzene received by workers who wore respirators could only be approximated by the air monitoring data. Therefore, total internal dose of benzene was characterised by the sum of the four metabolites in urine. As an alternative to normalising the concentrations of individual metabolites to the total metabolites, individual metabolites were calculated as a ratio to phenol. Others have used this approach[23] for normalising the urinary concentrations of other metabolites from exposure to benzene. This analysis led to essentially the same conclusions as with total metabolites. Phenol excretion has been shown to be linear with breath zone concentrations of benzene between 10 and 200 ppm as a time weighted average,[24] although the scatter in these data sets may not have distinguished any subtle shifts in metabolism. Although we found that the relative amount of total metabolites as indicated by phenol increased in more highly exposed workers, as noted previously, this overall increase was small.

We note that the compositional nature of our outcome variable may have increased the possibility of artifactual or chance findings. If the ratio of one metabolite to total metabolites increases, then the relative amount of one or more other metabolites to total metabolites must necessarily decrease. If the four measured metabolites comprised almost all the metabolites of benzene in urine, then conclusions drawn from relative increases or decreases in metabolite ratios would be appropriate. If, however, the decrease in one metabolite resulted in the formation of a second, unmeasured metabolite, then relative changes found could be, in part, artifactual. This is unlikely because, as previously noted, studies in rodents have shown that these metabolites, as their conjugates, represent the overwhelming majority of the metabolites found in urine.[5][6] Also, we had strong initial hypotheses, based on previous animal and theoretical studies,[9][14][23][25–27] that the proportion of total benzene metabolites made up of one or more of the toxic benzene metabolites would decrease as internal dose increased. The consistency between these previous observations and the data reported here reduces the probability that the results from this study are artifactual or chance observations.

Benzene metabolism has been extensively studied in various animal models, in vitro incubations, and by theoretical calculations.[9][14][23][25–27] The exact pathways and enzymatic systems involved remain unclear (fig 4). However, the trends found in our study are consistent with the model previously described by Schlosser *et al*,[9] based on benzene and phenol metabolism by mouse and rat liver microsomes. In this model, all of the metabolic steps were assumed to be catalysed by the same set of enzymes, probably cytochrome *P*-450. Furthermore, benzene and its ring hydroxylated metabolites were all assumed to reversibly compete for the same reaction site on the cytochrome. Competitive binding would suggest that as benzene concentration increases, metabolism of phenol to hydroquinone should decrease. Therefore, the ratio of hydroquinone to phenol would decrease. In support of the model, at higher concentrations, benzene is known to inhibit metabolism of phenol.[28] Our data for the human excretion of hydroquinone relative to phenol, as well as total metabolites, are consistent with this model.

At low benzene concentrations (μM) the model described by Schlosser *et al*[9] predicts that all of the catechol produced could be accounted for by the hydroxylation of phenol. In fact, rats given phenol intraperitoneally (50 mg/kg) have been shown to excrete increased concentrations of catechol.[29] Other studies suggest that at high benzene concentrations (mM), catechol is formed by the hydrolysis of the initial benzene metabolite, benzene oxide, to dihydrodiol (2 and 5, respectively, in fig 4), mediated by epoxide hydrolase. The dihydrodiol undergoes subsequent enzymatic rearomatisation to catechol.[30] Analogous to hydroquinone, if catechol was produced solely by hydroxylation of phenol, in vivo conversion of phenol to catechol would decrease with in-creasing exposure to benzene. However, the opposite was found in our study. These results suggest that at higher benzene concentrations the alternate pathway to catechol formation becomes considerable, increasing the ratio of catechol to phenol and total metabolites.

Muconic acid, which is thought to be indicative of the formation of the toxic intermediate muconaldehyde (9 in fig 4), has been found in only trace amounts in most in vitro incubations. Effective models for its formation have thus been difficult to develop. However, calculations suggest that muconaldehyde may be formed from oxepin in a two step process. In the first step, oxepin undergoes enzymatic epoxidation of oxepin (7 in fig 4), an equilibrium product of benzene oxide, to transoid-2,3-epoxyoxepin (8 in fig 4).[27] This intermediate spontaneously rearranges to muconaldehye, and would have a short half life in biological systems (<1 minute). Oxepin would be expected to compete for the same active site in the cytochrome as does benzene and phenol. As stated earlier, as benzene concentration increases, benzene oxide and oxepin would increase. However, competitive binding by benzene and phenol would inhibit conversion to the epoxyoxepin. Therefore, the benzene oxide would chemically rearrange to phenol, or be hydrolysed to benzene diol, rather than be converted to muconaldehyde and ultimately muconic acid. Consistent with our in vivo observation, the ratio of muconic acid to phenol would be expected to decrease with increasing exposure to benzene.

The relative contribution that each benzene metabolite makes to the adverse health effects of benzene, both alone and in various combinations, is still a subject of some debate. Several studies have shown that benzene metabolites related to muconic acid and hydroquinone are more genotoxic than phenol or compounds related to catechol.[31–35] In particular, growing evidence suggests that metabolites derived from hydroquinone, including 1,2,4-benzenetriol and particularly 1,4-benzoquinone,[36–38] are highly toxic.[10–13] However, other studies have shown synergistic effects between combinations of hydroquinone and other metabolites including phenol and catechol.[39–41] Thus, although the latest evidence points to hydroquinone having a central role in benzene toxicity, a role for other metabolites cannot be ruled out.

Urinary metabolites do not necessarily reflect metabolite concentrations in target tissues. However, the use of urinary metabolites of benzene to gain insight into the in vivo metabolism of benzene is supported by the finding that 87% of metabolites derived from benzene are excreted in the urine of rodents.[5] If we assume that the concentrations of benzene metabolites in urine reflect concentrations encountered in relevant tissues, our results suggest that the risk for adverse health outcomes due to exposure to benzene may have a complex relation with level of exposure. In particular, if the most important toxic benzene metabolites are assumed to derive from hydroquinone, ring opened precursors to muconic acid, or both, the results presented here suggest

that risk for adverse health outcomes due to benzene may show a supralinear relation with external dose. Indeed, we have recently reported that there was a proportionally smaller increase in risk for myelodysplastic syndromes and acute non-lymphocytic leukaemia with exposure to increasing concentrations of benzene in a retrospective cohort study of 74 828 workers exposed to benzene in China.[3] This dose-response relation was particularly evident for the 22 cases identified from a large subcohort of workers who were exposed to constant (fixed) concentrations of benzene over their working lifetimes.[3]

In summary, as exposure to benzene increased among workers exposed to a wide range of benzene concentrations in Shanghai, China, the proportion of benzene metabolites excreted as muconic acid and hydroquinone decreased whereas the proportion excreted as catechol and phenol increased. These complex patterns suggest that linear extrapolation of the toxic effects of benzene from highly exposed workers to less exposed populations may be inappropriate, to the extent that compounds related to hydroquinone and muconic acid play critical parts in benzene toxicity, and could lead to an underestimation of risk.

1 International Agency for Research on Cancer. Benzene. In: *International agency for research on cancer monographs on the evaluation of carcinogenic risks to man 29.* Lyon: IARC, 1982:93–148.

2 Yin SN, Hayes RB, Linet MS, *et al*. A cohort study of cancer among benzene-exposed workers in China: overall results. *Am J Ind Med* 1996;**29**:227–35.

3 Hayes RB, Yin S-N, Dosemeci M, *et al*. Benzene and the dose-related incidence of haematologic neoplasms in China. *J Natl Cancer Inst* 1997;**89**:1065–71.

4 Rinsky RA, Smith AB, Hornung R, *et al*. Benzene and leukemia. An epidemiologic risk assessment. *N Engl J Med* 1987;**316**:1044–50.

5 Sabourin PJ, Chen BT, Lucier G, *et al*. Effect of dose on the absorption and excretion of [^{14}C]benzene administered orally or by inhalation in rats and mice. *Toxicol Appl Pharmacol* 1987;**87**:325–36.

6 Sabourin PJ, Bechtold WE, Birnbaum LS, *et al*. Difference in the metabolism of inhaled [^{3}H] benzene by F344/N rats and B6C3F1 mice. *Toxicol Appl Pharmacol* 1988;**94**:128–40.

7 Sabourin PJ, Bechtold WE, Griffith WC, *et al*. Effect of exposure concentration, exposure rate, and route of administration on metabolism of benzene by F344 rats and B6C3F$_1$ mice. *Toxicol Appl Pharmacol* 1989;**99**:421–44.

8 Sabourin PJ, Sun JD, Birnbaum LS, *et al*. Effect of repeated benzene inhalation exposures on subsequent metabolism of benzene. *Exp Pathol* 1989;**37**:1557.

9 Schlosser PM, Bond JA, Medinsky MA. Benzene and phenol metabolism by mouse and rat liver microsomes. *Carcinogenesis* 1993;**14**:2477–86.

10 Levay G, Bodell WJ. Potentiation of DNA adduct formation in HL-60 cells by combinations of benzene metabolites. *Proc Natl Acad Sci USA* 1992;**89**:7105–9.

11 Irons R D, Stillman WS, Calagiovanni DG, *et al*. Synergistic action of the benzene metabolite hydroquinone on myelopoietic stimulating activity of granulocyte/macrophage colony-stimulating factor in vitro. *Proc Natl Acad Sci USA* 1992;**89**:3691–5.

12 Irons RD, Stillman WS. Cell proliferation and differentiation in chemical leukaemogenesis. *Stem Cells* 1993;**11**:235–42.

13 Zhang L, Wang Y, Shang N, *et al*. Benzene metabolites induce the loss and long arm deletion of chromosomes 5 and 7 in human lymphocytes. *Leukemia Res* 1998;**22**:105–13.

14 Snyder R, Kalf GF. A perspective on benzene leukaemogenesis. *Crit Rev Toxicol* 1994;**24**:177–209.

15 Rothman N, Li GL, Dosemeci M, *et al*. Hematotoxicity among Chinese workers heavily exposed to benzene. *Am J Ind Med* 1996;**29**:236–46.

16 Rothman N, Smith MT, Hayes RB, *et al*. An epidemiologic study of early biologic effects of benzene in Chinese workers. *Environ Health Perspect* 1996;**104**(suppl 6):1365–70.

17 Zhang L, Rothman N, Wang Y, *et al*. Interphase cytogenetics of workers exposed to benzene. *Environ Health Perspect* 1996;**104**(suppl 6):1325–9.

18 Rothman N, Haas R, Hayes RB, *et al*. Benzene induces gene-duplicating but not gene-inactivating mutations at the glycophorin A locus in exposed humans. *Proc Natl Acad Sci USA* 1995;**92**:4069–73.

19 Zhang L, Rothman N, Wang Y, *et al*. Benzene exposure is associated with increased aneusomy and long arm deletion of chromosomes 5 and 7 in Chinese workers. Submitted.

20 Bechtold WE, Lucier G, Birnbaum LS, *et al*. Muconic acid determinations in urine as a biological exposure index for workers occupationally exposed to benzene. *Am Ind Hyg Assoc J* 1991;**52**:473–8.

21 American Conference of Governmental Industrial Hygienists. *Documentation of the threshold limit values and the biological indices*. Cincinnati, OH: ACGIH, 1986.

22 Cary, NC: SAS Institute, 1987.

23 Sabourin PJ, Medinsky MA, Bechtold WE, *et al*. Disposition of benzene in animals and man. In: Mehlman MA, ed. *Advances in modern environmental toxicology, XVI. Benzene: occupational and environmental hazards.* Princeton, NJ: Princeton Scientific, 1990:153–76.

24 Inoue O, Seiji K, Kashara M, *et al*. Quantitative relation of urinary phenol levels to breath zone benzene concentrations: a factory survey. *Br J Ind Med* 1986;**43**:692–7.

25 Kalf GF. Recent advances in the metabolism and toxicity of benzene. *CRC Crit Rev Toxicol* 1987;**18**:141–59.

26 Goon D, Cheng XQ, Ruth JA, *et al*. Metabolism of trans, trans-muconaldehyde by aldehyde and alcohol dehydrogenases—identification of a novel metabolite. *Toxicol Appl Pharmacol*, 1992;**114**:147–55.

27 Greenberg A, Bock CW, George P, *et al*. Energetics of the metabolic production of (E,E)-muconaldehyde from benzene via the intermediates 2,3-epoxyoxepin and (Z,Z)-muconaldehyde and (E,Z)-muconaldehyde—ab initio molecular orbital calculations. *Chem Res Toxicol* 1993;**6**:701–10.

28 Koop DR, Laethem CL, Schnier GG. Identification of ethanol-inducible P450 isozyme 3a (P450IIE1) as a benzene and phenol hydroxylase. *Toxicol Appl Pharmacol* 1989;**98**:278–88.

29 Inoue O, Seiji K, Nakatsuka H, *et al*. Excretion of 1,2,4-benzenetriol in the urine of workers exposed to benzene. *Br J Ind Med* 1989;**46**:559–65.

30 Billings RE. Mechanisms of catechol formation from aromatic compounds in isolated rat hepatocytes. *Drug Metab Dispos* 1985;**13**:287–90.

31 Irons RD, Neptun DA. Effects of the principal hydroxymetabolites of benzene on microtubule polymerization. *Arch Toxicol* 1980;**45**:297–305.

32 Rushmore T, Snyder R, Kalf G. Covalent binding of benzene and its metabolites to DNA in rabbit bone marrow mitochondria in vitro. *Chem Biol Interact* 1984;**49**:133–54.

33 Schwartz CS, Snyder R, Kalf GF. The inhibition of mitochondrial DNA replication in vitro by the metabolites of benzene, hydroquinone and p-benzoquinone. *Chem Biol Interact* 1985;**53**:327–50.

34 Yager JW, Eastmond DA, Robertson ML, *et al*. Characterization of micronuclei induced in human lymphocytes by benzene metabolites. *Cancer Res* 1990;**50**:393–9.

35 Snyder R, Dimitriadis E, Guy R, *et al*. Studies on the mechanism of benzene toxicity. *Environ Health Perspect* 1989;**82**:31–5.

36 Ross D. Metabolic basis of benzene toxicity. *Eur J Haemotol* 1996;**57**(suppl):111–8.

37 Smith MT. The mechanism of benzene-induced leukemia: a hypothesis and speculations on the causes of leukemia. *Environ Health Perspect* 1996;**104**:1219–25.

38 Smith MT, Yager JW, Steinmetz KL, *et al*. Peroxidase-dependent metabolism of benzene's phenolic metabolites and its potential role in benzene toxicity and carcinogenicity. *Environ Health Perspect* 1989;**82**:23–9.

39 Robertson ML, Eastmond DA, Smith MT. Two benzene metabolites, catechol and hydroquinone, produce a synergistic induction of micronuclei and toxicity in cultured human lymphocytes. *Mutat Res* 1991;**249**:201–9.

40 Barale R, Marrazzini A, Betti C, *et al*. Genotoxicity of two metabolites of benzene, phenol, and hydroquinone show strong synergistic effects in vivo. *Mutat Res* 1990;**244**:15–20.

41 Chen H. Eastmond DA. Synergistic increase in chromosomal breakage within the euchromatin induced by an interaction of the benzene metabolites phenol and hydroquinone in mice. *Carcinogenesis* 1995;**16**:1963–9.

Urinary excretion of phenol, catechol, hydroquinone, and muconic acid by workers occupationally exposed to benzene.

N Rothman, W E Bechtold, S N Yin, et al.

Updated information and services can be found at:

http://oem.bmj.com/content/55/10/705

These include:

References	Article cited in: http://oem.bmj.com/content/55/10/705#related-urls
Email alerting service	Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:

http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:

http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:

http://group.bmj.com/subscribe/