Concentrations of urinary metabolites in workers exposed to monochlorobenzene and variation in the concentration during a workshift

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Abstract

Urinary concentrations of metabolites of monochlorobenzene were examined in 10 male workers exposed to the compound while synthesising intermediate products for dyes. Their individual exposure concentrations were monitored for the whole workshift and samples of urine were collected at the start and end of the workshift, during it, and during the noon recess. The concentrations of four metabolites, 4-chlorocatechol and *o*-, *m*-, and *p*-chlorophenol, in the urine samples were measured. The investigation was performed on Monday and Tuesday in one week and on Tuesday and Wednesday in another week. The concentrations of 4-chlorocatechol in urine collected during the last four hours and at the end of the workshift were proportional to the eight hour time weighted average exposure to monochlorobenzene. The concentration in urine collected during the noon recess showed a linear correlation with the four hour time weighted average in the morning. Similarly, linear relations were obtained for urinary *p*-chlorophenol. The ratio (as monochlorobenzene) of *p*-chlorophenol to 4-chlorocatechol concentrations at the start of the workshift was 0.39 and at the end of the workshift was 0.22. The ratios of the urinary concentrations 15 hours after exposure to those at the end of exposure were 0.24 for 4-chlorocatechol and 0.44 for *p*-chlorophenol. The present study also showed that variations in exposure at the workplace were reflected by changes in concentrations of urinary metabolites during the workshift.

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Monochlorobenzene is a colourless compound widely used as an organic solvent, as a chemical intermediate, and as a heat transfer medium. Animal studies have shown that it has adverse effects on the liver and kidney.¹⁻³ In humans, monochlorobenzene is metabolised to 4-chlorocatechol, *o*-, *m*-, and *p*-chlorophenols, and 4-chlorophenylmercapturic acid.^{4,5} In an experimental study on volunteers exposed to monochlorobenzene, Ogata *et al* found linear correlations between airborne exposure and concentrations of urinary 4-chlorocatechol and *p*-chlorophenol.⁶

Yoshida *et al* found similar results with workers exposed to monochlorobenzene.⁵ These results suggest that concentrations of urinary 4-chlorocatechol and *p*-chlorophenol are good biological monitoring indices. The slope of the regression line of the concentration of urinary 4-chlorocatechol against the exposure estimated by Yoshida *et al* was three times higher than that of Ogata *et al*. Thus our study was carried out to re-examine the relation between exposure to airborne monochlorobenzene in the workplace and the concentration of urinary metabolites.

We also examined how the concentration of urinary metabolites vary during a workshift. As the exposure usually varies with time at the workplace, the concentration of urinary metabolite must also be affected. We investigated the relation with time of the exposure concentration and that of the urinary metabolites.

Subjects and methods

WORKERS STUDIED

Ten male employees in a plant synthesising intermediate products for dyes participated in this study. They were exposed to the solvents monochlorobenzene and *o*-dichlorobenzene from 0800 to 1700 with a one hour noon recess. Their ages ranged between 38 and 60 years.

CHEMICALS

All reagents and chemicals were special or first grade, obtained from Tokyo Kasei Ind (Tokyo, Japan), Kanto Chemical Co (Tokyo, Japan), and Merck (Darmstadt, Germany).

AIR SAMPLING AND ANALYSIS

Individual exposure to monochlorobenzene at the workplace was monitored with a passive dosimeter (Pro-Tec AA, Pro-Tec Systems Inc, Portland, USA). Samples were collected in the breathing zone from 0800 to 1200 and from 1300 to 1700. In the case of two workers, the passive dosimeter was exchanged every hour. For analysis, the carbon felt in the dosimeter was steeped in CS₂ for one hour and the solvent was injected into a gas chromatograph (HP 5890 series II, Hewlett Packard, USA) connected to a capillary column, DB-1 (15 m × 0.53 mm internal diameter, Jand W Scientific, Folsom, USA). Helium was used as the carrier gas at a flow rate of 7.5 ml/min. The temperature of the oven was 100°C, the injector and the detector 150°C.

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Figure 1 High performance liquid chromatograms of chlorobenzene metabolites in a standard and a urine sample.

URINE COLLECTION AND ANALYSIS

All samples of urine were collected during the workshift, including samples at its start, during the noon recess, and at the end. The volume and gravity of the samples of urine were measured, and then they were stored at -30°C until the analysis. For analysis, a 5 ml sample was placed in a glass tube (10 ml volume) with a screw cap, and 1.5 ml of concentrated hydrochloric acid was added. To hydrolyse conjugated metabolites, the mixture was heated for three hours in an oil bath (100°C). When the mixture had cooled, 2.0 ml of diethyl ether was added. The sample

was shaken vigorously for 10 minutes and centrifuged at 3000 rpm for 10 minutes. A 0.5 ml portion of the diethyl ether layer was placed in a glass tube and 2.0 ml of methyl alcohol was added. Next, 20 μl of this mixture was injected into a high performance liquid chromatograph (HPLC 6000 series, Hitachi, Tokyo, Japan) equipped with a stainless steel column (125 mm $\times$ 4 mm internal diameter) packed with RP-select B (Merck, Darmstadt, Germany). The column temperature was 30°C and the mobile phase was 70% methyl alcohol supplied at a flow rate of 1.0 ml/min. The effluents of 4-chlorocatechol and *o*-, *m*- and *p*-chlorophenols were monitored at 280 nm. For calibration curves 50.0 to 500 mg/l of standard solution of 4-chlorocatechol and 6.2 to 63.4 mg/l of *o*-, *m*-, and *p*-chlorophenols were treated as described.

The investigation was performed on Monday and Tuesday in one week and on Tuesday and Wednesday in another week. The total number of monitored working days was 37.

Results

ACCURACY OF URINE ANALYSIS

Figure 1 shows HPLC chromatograms for a standard and a sample. Peaks of 4-chlorocatechol and *o*-, *p*-, and *m*-chlorophenols were identified by their retention times. All calibration curves had good linearity (fig 2).

When control urine specimens were spiked at concentrations of 100, 300, and 500 mg/l, the mean recovery rates of 4-chlorocatechol were 96.2% to 98.2% (coefficient of variation (CV) 0.8% to 2.1%, $n = 3$ for each concentration). The mean recovery rates of *o*-, *m*-, and *p*-chlorophenols were 99.6% to 101% (CV 0.2% to 1.2%, $n = 3$) at 12.4, 37.1, and 61.8 mg/l, 104% to 107% (CV 1.0% to 1.6%, $n = 3$) at 12.7, 38.0, and 63.4 mg/l, and 99.3% to 101% (CV 1.0% to 1.9%, $n = 3$) at 12.6, 37.8, and 63.0 mg/l. Figure 3 shows the effect of heating time (zero to five hours) on the acid hydrolysis of conjugated metabolites. There was no change in hydro-

Figure 2 Calibration curves of 4-chlorocatechol and *o*-, *m*- and *p*-chlorophenols.

Figure 3 Rate of hydrolysis with heating time.

Table Variables of regression lines and correlation coefficients

Metabolite	Observed			Corrected for specific gravity (1.024)			Corrected for creatinine		
	a	b	r	a	b	r	a	b	r
4-Chlorocatechol (CC_{en})	13.0	12.4	0.86*	13.0	6.9	0.92*	7.30	7.86	0.97*
p-Chlorophenol ($p-CP_{en}$)	2.27	3.18	0.88*	2.31	1.92	0.94*	1.30	1.93	0.98*
$y = ax + b$									
$x = 8 \text{ h TWA}$									
$y = \text{metabolite concentration in urine collected at the end of the workshift}$									
4-Chlorocatechol (CC_{pm})	10.5	13.8	0.83*	12.7	3.9	0.94*	7.76	3.82	0.99*
p-Chlorophenol ($p-CP_{pm}$)	1.71	3.97	0.82*	2.12	1.78	0.94*	1.30	1.33	0.98*
$y = ax + b$									
$x = 8 \text{ h TWA}$									
$y = \text{metabolite concentration in urine collected during the last 4 hours of the workshift}$									
4-Chlorocatechol (CC_{no})	8.43	8.54	0.82*	10.4	4.3	0.88*	7.97	2.43	0.96*
p-Chlorophenol ($p-CP_{no}$)	1.36	2.48	0.75*	1.70	1.48	0.84*	1.31	0.78	0.94*
$y = ax + b$									
$x = 4 \text{ h TWA in the morning}$									
$y = \text{metabolite concentration in urine collected during the noon recess}$									

* $p < 0.001$. x expressed in ppm. y is expressed in mg/l for observed values and for values corrected for specific gravity and in mg/g creatinine for values corrected for creatinine concentration.

lysis from one to five hours. In this study, a heating time of three hours was chosen.

For o- and m-chlorophenols in a few cases, the urinary concentrations were less than the detection limit (0.1 mg/l) or the peak areas could not be calculated due to interference from unknown peaks; these metabolites are not discussed.

RELATION BETWEEN AIRBORNE EXPOSURE OF MONOCHLOROBENZENE AND CONCENTRATION OF URINARY METABOLITES

The long-term limit of time weighted average (TWA) exposure for monochlorobenzene is 230 mg/m³ in the United Kingdom and the threshold limit value (TLV) given by the American Conference of Governmental Hygienists (ACGH) is 46 mg/m³ in the United States. In the present study the 8 h TWA value of monochlorobenzene ranged from 0.2 to 38.5 ppm. For dichlorobenzene it ranged from >0.1 ppm to 4.5 ppm. Concentrations of 4-chlorocatechol in urine collected at the start of the workshift, during the noon recess, at the end of the workshift, and during the last four hours of the workshift were denoted by CC_{st} , CC_{no} , CC_{en} , and

CC_{pm} . Similarly, concentrations of p-chlorophenol were denoted by $p-CP_{st}$, $p-CP_{no}$, $p-CP_{en}$, and $p-CP_{pm}$. The table shows the variables of the regression lines and the correlation coefficients between the TWA value and the concentration of urinary metabolites. As the correlation coefficients were the highest for creatinine corrected values, these corrected values were chosen for the biological monitoring. Figures 4 and 5 show the good correlations between the 8 h TWA value and CC_{pm} , and between the 8 h TWA value and $p-CP_{pm}$. The 8 h TWA value also correlated linearly with CC_{en} and $p-CP_{en}$ (table). The slopes of the regression lines of CC_{pm} and CC_{en} were nearly equal. On the other hand, CC_{no} and $p-CP_{no}$ correlated linearly with the 4 h TWA value in the morning (table).

RATIOS BETWEEN CONCENTRATIONS OF URINARY METABOLITES

The mean ratio (as monochlorobenzene) of $p-CP_{st}$ to CC_{st} was 0.40 (SD = 0.16, $n = 15$, except for Monday because of non-exposure on the previous day) and that of $p-CP_{en}$ to CC_{en} was 0.22 (SD = 0.05, $n = 27$). The mean ratio of CC_{st} for the second day to CC_{en}

Figure 4 Relation between 8 h TWA value of monochlorobenzene and concentration of 4-chlorocatechol in urine collected during the last four hours of the workshift.

Figure 5 Relation between 8 h TWA value of monochlorobenzene and concentration of p-chlorophenol in urine collected during the last four hours of the workshift.

Figure 6 Relation between 4 h TWA value of monochlorobenzene in the morning and concentration of 4-chlorocatechol in urine collected during the noon recess.

for the first day was 0.24 (SD = 0.09, $n = 14$), and that for *p*-chlorophenol was 0.44 (SD = 0.08, $n = 11$).

VARIATION OF AIRBORNE EXPOSURE AND CONCENTRATIONS OF URINARY METABOLITES

Figure 7 shows, for worker A, the 1 h TWA value and the concentration of urinary 4-chlorocatechol with time. On the first day, the 1 h TWA for monochlorobenzene ranged from 0.2 to 42 ppm (mean (SD) 7.9 (12.5)

Figure 7 Concentration of monochlorobenzene exposure with time and concentration of urinary 4-chlorocatechol for worker A.

Figure 8 Concentration of monochlorobenzene exposure with time and concentration of urinary 4-chlorocatechol for worker B.

ppm). Because high exposure was found in the first half of the workshift, the concentration of urinary 4-chlorocatechol increased in the morning, reached 91 mg/g creatinine at the noon recess, and then decreased. On the second day the 1 h TWAs of monochlorobenzene ranged from 0.6 to 40.4 ppm (mean (SD) 8.5 (12.2) ppm). High exposure was found in the last part of the workshift, and concentration of urinary 4-chlorocatechol was highest at the end. Although the means (SD) of the 1 h TWAs were about the same for the two days, the patterns of the time series of the 1 h TWA were different and the profiles of variation of the concentration of urinary metabolites were also different. It is interesting that the concentrations of urinary 4-chlorocatechol were close to each other at the end of the workshifts on these two days of nearly equal 8 h TWA values.

Figure 8 shows, for worker B, the time series of exposure and concentrations of urinary 4-chlorocatechol. On the first day, the 1 h TWA values varied from 1.6 to 61 ppm (mean (SD) 21.2 (18.5) ppm). High exposure periods were seen in both the first and the second half of the workshift, and the concentrations of urinary 4-chlorocatechol increased continuously throughout the day. On the second day, the 1 h TWA values ranged from 0.4 to 20 ppm (mean (SD) 7.2 (6.8) ppm), with low exposure in the middle period. The concentrations of urinary 4-chlorocatechol increased in the morning shift and then slightly decreased. On the other hand, at the start of the workshift on the second day, the concentration of urinary 4-chlorocatechol was already about 60 mg/g creatinine because of high exposure on the previous day. The high starting level may explain why the concentration of 4-chlorocatechol at the end of the shift was higher in the urine of worker B than in the urine of worker A although their 8 h TWA exposure was the same on the second day.

Discussion

The present study showed that because CC_{pm} and CC_{en} were proportional to the 8 h TWA value of monochlorobenzene with correlation coefficients (r) higher than 0.95, the concentrations of urinary 4-chlorocatechol seem to be good biological exposure indices. The slope of the regression line of CC_{en} on 8 h TWA values was nearly equal to that of CC_{pm} . If the exposure concentration is constant over time, the concentration of urinary metabolite would increase with time and reach the maximum at the end of the workshift; the slope of the regression line would be highest for urinary concentrations at the end of the workshift. The exposure concentration usually varies over time and the concentration of urinary metabolites can decrease or increase during the workshift (fig 7 and 8). Thus the concentration of urinary metabolite is not necessarily highest at the end of the workshift. It should be noted, however, that both concentrations of urinary metabolite at

the end of the workshift and during the last four hours correlated linearly with the 8 h TWA value regardless of variation in the level of exposure during the workshift. We also found that both p -CP_{pm} and p -CP_m correlated linearly with the 8 h TWA value, and that the concentration of urinary p -chlorophenol can be a good biological exposure index.

Ogata *et al* reported that the CC_{pm} was 57 mg/g creatinine and CP_{pm} was 10 mg/g creatinine for five volunteers exposed to monochlorobenzene of 10 ppm at rest.⁶ Our values of 81 and 14 mg/g creatinine were slightly higher, perhaps because of the higher inhalation rate due to higher workload. Yoshida *et al* reported that CC_m in workers exposed to 10 ppm monochlorobenzene (8 h TWA) was 180 mg/g creatinine.⁵ In our study, the corresponding value was 81 mg/g creatinine, about half their value. This may be due to a difference in the workload or the pattern of exposures with time.

For the biological monitoring of monochlorobenzene, urine should be collected at the end of the workshift or during the last four hours, when the concentrations of metabolite reflect the daily average exposure. If this is difficult, collection during the noon recess may be the second best strategy, because CC_{no} and p -CP_{no} correlated linearly with the 4 h TWA value in the morning. This would not be suitable, however, if afternoon exposure differs greatly from that in the morning. It should be also noted that these concentrations of metabolite may reflect high exposure on the previous day.

The ratio (as monochlorobenzene) of concentrations of p -chlorophenol to 4-chlorocatechol was 0.22 for urine collected at the end of the workshift. This is close to the value (0.16) reported by Yoshida *et al*.⁵ However, the ratio depended on collection time. For urine collected at the start of the workshift, the ratio (0.39) was about twice the end of workshift ratio. This difference can be explained by the fact that the excretion rate of p -chlorophenol is slower than that of 4-chlorocatechol.⁶ Ogata *et al* estimated the elimination curves of 4-chlorocatechol and p -chlorophenol in their experimental study.⁶ The curves show that the ratios of the concentrations of urinary metabolites at 15 hours after exposure to those at the end of exposure were 0.07 for 4-chlorocatechol and 0.24 for

p -chlorophenol. These values were considerably lower than our corresponding values (0.24 and 0.44). This may be because the workers in our study were exposed repeatedly every day resulting in accumulation in fat tissue where blood flow rate is low and the fat tissue to blood partition coefficient is high.⁷⁻⁹ Consequently, once organic solvent accumulates in the fat tissue, it is released slowly. This suggests that the biological half life found in an experimental study in which volunteers are exposed only once to a chemical for eight hours would be considerably different from that in workers who are repeatedly exposed.

In our study, the concentrations of urinary 4-chlorocatechol and p -chlorophenol were confirmed to be good biological exposure indices of monochlorobenzene. The slopes of the regression line of the concentrations of urinary metabolites against the airborne exposures were considerably different from those estimated in past studies, perhaps because of differences in workloads and the patterns of exposure. We also found that variation of the airborne exposure caused the concentrations of urinary metabolites to increase or decrease during the workshift. With a physiologically based pharmacokinetic model, we are now investigating how the difference in workload and variation of the exposure affects the concentrations of urinary metabolites.

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