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Relations between Exposure to *o*-Dichlorobenzene and Concentrations of Urinary Metabolites

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Abstract: Relations between Exposure to *o*-Dichlorobenzene and Concentrations of Urinary Metabolites: Shinji KUMAGAI, *et al.* Department of Occupational Health, Osaka Prefectural Institute of Public Health—The present study aimed to examine the relation between exposure to airborne *o*-dichlorobenzene (*o*-DCB) and concentrations of four urinary metabolites, i.e., 3,4-dichlorocatechol (3,4-DCC), 4,5-dichlorocatechol (4,5-DCC), 2,3-dichlorophenol (2,3-DCP) and 3,4-dichlorophenol (3,4-DCP). Subjects were ten male workers exposed to *o*-DCB in synthesizing intermediate products for dyes. Individual exposure to *o*-DCB was monitored with a passive dosimeter. Urine samples were collected during the workshift and at the end of the workshift. The concentrations of urinary 3,4-DCC, 4,5-DCC, 2,3-DCP and 3,4-DCP were determined with a high performance liquid chromatograph. The mean recovery rates for urinary metabolites obtained by our analytical method were 93.2 to 102% with coefficients of variance of 2.7 to 7.5%. The concentrations of 3,4-DCC, 4,5-DCC, 2,3-DCP and 3,4-DCP in urine collected at the end of the workshift and those during the last four hours of the workshift were shown to be well correlated to the 8-hr time weighted average (8-hr TWA) value for the exposure concentration of *o*-DCB. The mean ratios (as moles) of 3,4-DCC to 4,5-DCC, that of 2,3-DCP and that of 3,4-DCP at the end of the workshift were 0.78, 0.42 and 0.37, respectively. The time series of the concentrations of the four metabolites showed that these concentrations varied with time in a similar pattern.
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Key words: *o*-dichlorobenzene, 3,4- and 4,5-dichlorocatechols, 2,3- and 3,4-dichlorophenols, Urinary metabolites, Biological monitoring

o-Dichlorobenzene (*o*-DCB) is a colorless liquid compound widely used as an organic solvent, as a chemical intermediate and as a heat transfer medium. It is also used as a deodorant and as an insecticide. This chemical has adverse effects on animals and humans[1,2]. A rat study showed that high exposure to *o*-DCB by inhalation introduced acute liver necrosis and kidney tubule damage[3]. Mice exposed to saturated *o*-DCB vapor showed prompt narcosis followed by central depression and cyanosis[4]. In rats and rabbits repeatedly exposed to *o*-DCB at 100 to 400 ppm, maternal toxicity was reflected by a reduced rate of body weight gain and liver weight was increased[5]. In humans, some irritation to the eyes and upper respiratory system was observed in workers exposed to *o*-DCB[1,6]. The exposure level should therefore be controlled at workplaces. In rabbits, it is metabolized to 3,4-dichlorocatechol (3,4-DCC), 4,5-dichlorocatechol (4,5-DCC), 2,3-dichlorophenol (2,3-DCP), 3,4-dichlorophenol (3,4-DCP) and 3,4-dichlorophenylmercapturic acid, and then excreted in urine[7]. Reid *et al*. suggested that arene oxides may be precursors of the excreted metabolites[8]. Previously we showed that *o*-DCB is metabolized to 3,4-DCC, 4,5-DCC, 2,3-DCP and 3,4-DCP in humans[9]. The present study aimed to develop a method for quantitatively determining these urinary metabolites and to examine the relation between exposure to airborne *o*-DCB and concentrations of urinary metabolites.

Subjects and Methods

Workers studied

Ten male workers were employed in a plant synthesizing intermediate products for dyes and all of the workers participated in this study. They were exposed to *o*-DCB used as solvent. The workshift was from 8:00 to 17:00 with one hour noon recess. The ages of the subjects ranged between 38 and 60 years (mean (SD) = 51.7 years (6.4 years)). The survey was conducted on three days and 28 person-day samples were obtained.

Chemicals

2,3-DCP and 3,4-DCP were obtained from Tokyo Kasei (Tokyo, Japan), 3,4-DCC from Helix Biotech (Lichmond, Canada). 4-chlorocatechol and sulfuryl chloride were

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purchased from Tokyo Kasei and Wako Pure Chemical (Osaka, Japan), respectively. All chemicals were special or first grade. 4,5-DCC was synthesized by reacting 4-chlorocatechol with sulfuryl chloride in diethyl ether, evaporating ether and recrystallizing from carbon disulfide. The melting point was 116–117°C, which was the same as reported by Willstätter *et al.*[10].

Air sampling and analysis

Individual exposure to *o*-DCB at the workplace was monitored with a passive dosimeter (Pro-Tec AA, Pro-Tec Systems Inc., Portland, USA). In the case of two workers (A and B), the passive dosimeter was changed every hour to obtain a time series of exposure concentrations. For analysis, the carbon felt in the dosimeter was steeped in carbon disulfide for one hour and the solvent was injected into a gas chromatograph (HP 5890 series II, Hewlett Packard, USA) supplied by a capillary column, DB-1 (15 m × 0.53 mm I.D., J & W Scientific, Folson, USA). Helium was used as the carrier gas at a flow rate of 7.5 m*l*/min. The temperatures of the oven, injector and detector were 100°C, 150°C and 150°C, respectively.

Urine collection and analysis

All urine samples were collected during the workshift, including samples at the start of the workshift, during the noon recess and at the end of the workshift. The volume and gravity of the urine samples were measured, and the samples were stored at −30°C until the analysis. All urine samples from workers A and B and urine samples collected from the other workers during the last 4 hr were used to determine the concentrations of metabolites. For analysis, a 5 m*l* urine sample was placed in a glass tube (10 m*l* volume) with a screw cap, and 1.5 m*l* of concentrated hydrochloric acid was added. To hydrolyze conjugated metabolites, the mixture was heated for three hours in an oil bath (100°C). When the mixture had cooled, 2.0 m*l* of diethyl ether was added. The sample was shaken vigorously for ten minutes and centrifuged at 3,000 rpm for ten minutes. A 0.5 m*l* of the ethyl ether layer was separated into a glass tube, and 2.0 m*l* of methyl alcohol was added. Next, 20 μl of the mixture was injected into a high performance liquid chromatograph (HPLC 6000 series, Hitachi, Tokyo, Japan). For calibration curves, 9.0 to 90 mg/*l* of water solution of 3,4-DCC and 10.0 to 100.0 mg/*l* of 4,5-DCC, 2,3-DCP and 3,4-DCP were treated as above without heating. The HPLC was equipped with a stainless-steel column (125 mm × 4 mm I.D.) packed with LiChrospher 100 RP-18(e) (Merck, Darmstadt, Germany). The column temperature was 30°C. For 3,4-DCC and 4,5-DCC, the mobile phase was a mixture of acetonitrile, acetic acid and water (1:12:87) supplied at a flow rate of 2.0 m*l*/min (Method I). For 2,3-DCP and 3,4-DCP, the mobile phase was a mixture of methyl alcohol, acetic acid and water (33:5:62) supplied at a flow rate of 1.5 m*l*/min (Method II). The effluents of dichlorophenols were monitored at 285 nm. The concentration of urinary creatinine was determined by the method of Jaffe[11].

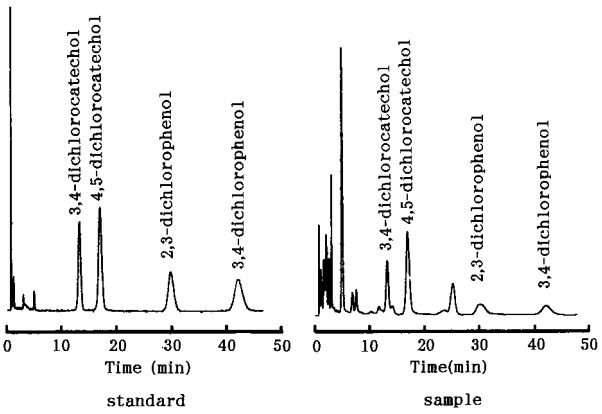

Fig. 1. High performance liquid chromatograms of *o*-dichlorobenzene metabolites in a standard and a urine sample in analytical method I.

Results

Accuracy of urine analysis

Figure 1 shows HPLC-chromatograms for Method I. Of the peaks on the chromatogram obtained from a sample, four peaks were identified as 3,4-DCC, 4,5-DCC, 2,3-DCP and 3,4-DCP by their retention times. But unknown peaks interfered with the peaks of DCPs in many cases. Figure 2 shows HPLC-chromatograms for Method II. Two peaks of the sample were identified as 2,3-DCP and 3,4-DCP by their retention times, because these peaks were not overlapped by unknown peaks. But the peaks of DCCs were overlapped. Consequently, DCCs and DCPs were analyzed by Methods I and II, respectively, but in six cases for 3,4-DCC and in three cases for 2,3-DCP, the peak areas could not be calculated due to interference from unknown peaks. The calibration curves of 3,4- and 4,5-DCCs (Method I) and 2,3- and 3,4-DCPs (Method II) were shown to have good linearity (Fig. 3). Detection limits were 0.5 mg/*l* for the four metabolites.

When control urine was spiked at concentrations of 9, 18 and 36 mg/*l*, the mean recovery rates of 3,4-DCC were 94.6 to 102% (CV=2.8 to 4.3%, n=6 for each concentration). The mean recovery rates of 4,5-DCC, 2,3-DCP and 3,4-DCP were 94.1 to 97.5% (CV=3.0 to 7.5%, n=6) at 10, 20 and 40 mg/*l*, 94.8 to 97.2% (CV=2.8 to 5.0%, n=6) and 93.2 to 95.8% (CV=2.7 to 5.8%, n=6), respectively. In order to investigate the effect of heating time on the acid hydrolysis of conjugated metabolites, heating times of zero, one, three and five were tested. The rate of hydrolysis was the highest at three hours, so three hours was chosen as the heating time in this study.

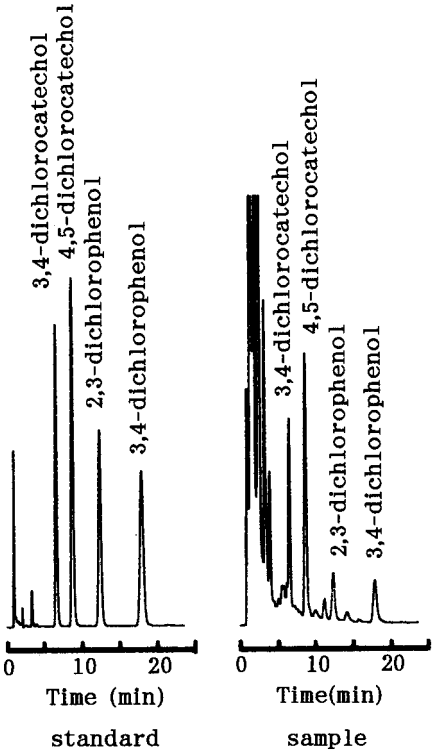

Fig. 2. High performance liquid chromatograms of *o*-dichlorobenzene metabolites in a standard and a urine sample in analytical method II.

Relation between airborne exposure to o-DCB and concentrations of urinary metabolites

Dichlorocatechol concentrations in urine collected at the end of the workshift and during the last 4 hr of the workshift were denoted by DCC_{en} and DCC_{pm}, respectively. Similarly, dichlorophenol concentrations were denoted by DCP_{en} and DCP_{pm}. Figure 4 shows the relationships of DCC_{en} and DCP_{en} to 8-hr time-weighted average (8-hr TWA) values for the exposure concentration of *o*-DCB. The parameters of the regression lines and the correlation coefficients between the TWA value and concentrations of urinary metabolites are listed in Tables 1 and 2. Figure 4 shows that TWA values for exposure concentration of *o*-DCB dispered from 0.1 to 2.3 ppm and that 3,4-DCC_{en} and 4,5-DCC_{en} correlated to the 8-hr TWA value, and 2,3-DCP_{en} and 3,4-DCP_{en} correlated to the 8-hr TWA value (Table 1). Similarly, Table 2 shows good correlation of 3,4-DCC_{pm}, 4,5-DCC_{pm}, 2,3-DCP_{pm} and 3,4-DCP_{pm} to the 8-hr TWA value. Because the correlation coefficients were the highest for creatinine corrected values except for 4,5-DCC_{en}, the corrected value was appropriate for the biological monitoring. The mean ratios (as moles) of 3,4-DCC_{en} to 4,5-DCC_{en}, that of 2,3-DCP_{en} and that of 3,4-DCP_{en} were 0.78 (SD=0.32), 0.42 (SD=0.19) and 0.37 (SD=0.18), respectively.

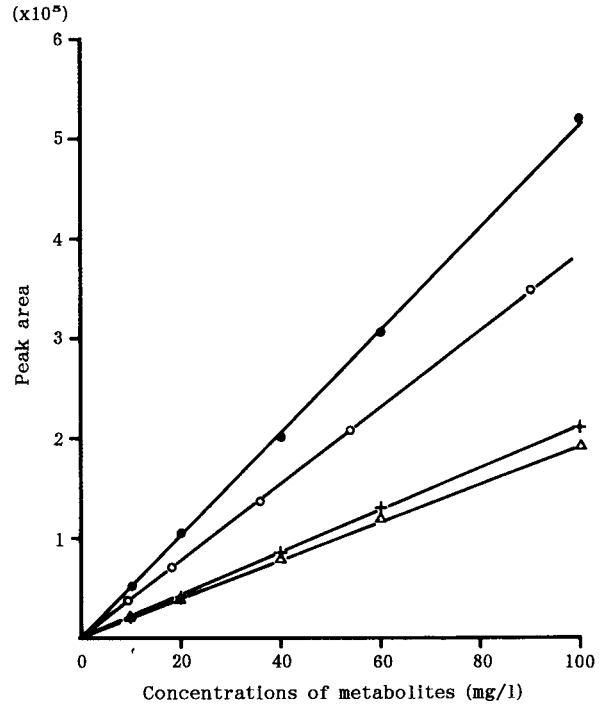

Fig. 3. Calibration curves of 3,4- and 4,5-dichlorocatechols, and 2,3- and 3,4-dichlorophenols. ○: 3,4-dichlorocatechol, ●: 4,5-dichlorocatechol, △: 2,3-dichlorophenol, +: 3,4-dichlorophenol.

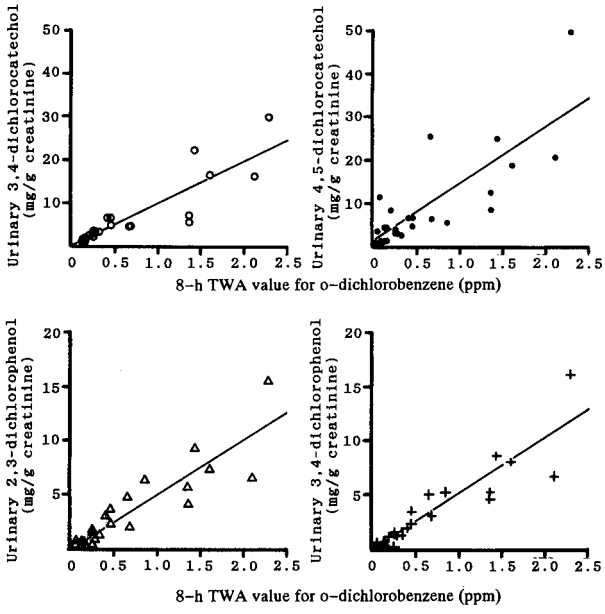

Fig. 4. Relation between 8 hr TWA value for *o*-dichlorobenzene and concentrations of 3,4- and 4,5-dichlorocatechols, and 2,3- and 3,4-dichlorophenols in urine collected at the end of the workshift.

Table 1. Variables of regression lines and correlation coefficients for metabolite concentrations in urine collected at the end of the workshift

		Observed			Corrected for specific gravity (1.024)			Corrected for creatinine		
Metabolite	n	a	b	r	a	b	r	a	b	r
3,4-dichlorocatechol	22	−0.41	17.4	0.84*	0.52	14.1	0.86*	0.03	9.84	0.88*
4,5-dichlorocatechol	28	0.38	24.0	0.79*	1.19	19.7	0.83*	1.02	13.4	0.81*
2,3-dichlorophenol	25	−0.58	8.69	0.86*	0.00	7.27	0.90*	−0.11	5.03	0.90*
3,4-dichlorophenol	28	−0.55	8.98	0.86*	−0.12	7.57	0.91*	−0.18	5.21	0.92*

1) x=8 hr TWA (ppm), 2) y=urinary metabolite concentration (expressed in mg/*l* for observed values and for values corrected for specific gravity and in mg/g creatinine for values corrected for creatinine), 3) a: intercept, b: slope, r: correlation coefficient, 4) *: $p<0.001$.

Table 2. Variables of regression lines and correlation coefficients for metabolite concentrations in urine collected during the last four hours of the workshift

		Observed			Corrected for specific gravity (1.024)			Corrected for creatinine		
Metabolite	n	a	b	r	a	b	r	a	b	r
3,4-dichlorocatechol	22	−1.71	19.1	0.83*	−0.60	16.5	0.88*	−0.63	11.4	0.91*
4,5-dichlorocatechol	28	−1.82	27.3	0.77*	−0.64	23.6	0.85*	−0.19	15.9	0.86*
2,3-dichlorophenol	25	−1.02	9.36	0.83*	−0.40	8.25	0.88*	−0.32	5.65	0.90*
3,4-dichlorophenol	28	−0.92	9.76	0.83*	−0.41	8.61	0.89*	−0.32	5.87	0.91*

1) x=8 hr TWA (ppm), 2) y=urinary metabolite concentration (expressed in mg/*l* for observed values and for values corrected for specific gravity and in mg/g creatinine for values corrected for creatinine), 3) a: intercept, b: slope, r: correlation coefficient, 4) *: $p<0.001$.

Variation in airborne exposure and urinary metabolite levels

Figure 5 shows the 1-hr TWA for *o*-DCB and the concentrations of urinary metabolite with time for worker A. The 1-hr TWAs for *o*-DCB ranged from 0.1 to 7.9 ppm (mean (SD)=2.1 ppm (2.5 ppm)). Because high exposure was found in the first half of the workshift, the concentrations of urinary 3,4-DCC, 4,5-DCC, 2,3-DCP and 3,4-DCP increased in the morning, reached 31, 42, 12 and 13 mg/g creatinine, respectively, at the noon recess, and then decreased. Figure 6 shows the time series of exposure and concentrations of urinary metabolites for worker B. The 1-hr TWA values varied from 0.1 to 8.8 ppm (mean (SD)=1.4 ppm (2.9 ppm)). A high exposure period was seen in the first half of the workshift, and the concentrations of urinary 3,4-DCC, 4,5-DCC, 2,3-DCP and 3,4-DCP increased with time, reached 16, 20, 6.6 and 7.7 mg/g creatinine, respectively, at 15:00, and then decreased. These two examples showed that the four urinary metabolite levels varied in a similar pattern.

Discussion

Oxidative hydroxylation and mercapturic acid formation are the main routes of conversion for chlorinated benzenes[12]. In rabbits given oral doses of *o*-DCB, 3,4-DCP in approximately 30% of the dose, 2,3-DCP in 9%, and 3,4- and 4,5-DCCs in 4% were observed to be excreted in the urine[7]. The present study showed that, in humans, *o*-DCB is metabolized to 4,5-DCC in the largest amount, 3,4-DCC in the second, and 2,3-DCP and 3,4-DCP in the smallest. From the mean ratios for the four metabolites, the amounts of 3,4-DCC, 4,5-DCC, 2,3-DCP and 3,4-DCP were calculated to be 30, 39, 16 and 15%, respectively, of the total amount of these four metabolites in the urine collected at the end of the workshift. There is therefore a considerable difference between humans and animals in the metabolism profile. In the above rabbit study[7], 3,4-dichlorophenylmercapturic acid was also identified as a minor component in the urine sample. In this study, this metabolite was not examined because the authentic standard could not be obtained.

This study showed that $3,4\text{-DCC}_{en}$, $4,5\text{-DCC}_{en}$, $2,3\text{-DCP}_{en}$ and $3,4\text{-DCP}_{en}$ were approximately proportional to the 8-hr TWA value for *o*-DCB. These urinary levels therefore seem to be good biological indicators of daily average exposure to *o*-DCB. We also found that $3,4\text{-DCC}_{pm}$, $4,5\text{-DCC}_{pm}$, $2,3\text{-DCP}_{pm}$ and $3,4\text{-DCP}_{pm}$ correlated well with the 8-hr TWA value, and that these concentrations can be good biological indicators of exposure to *o*-DCB. However, in a few cases, the chromatographic peaks of 3,4-DCC and 2,3-DCP were overlapped by unknown peaks in our analytical conditions, so that a better analytical method must be developed for these metabolites. In a comparison between 4,5-DCC and 3,4-DCP, because the slope of the regression line of urinary

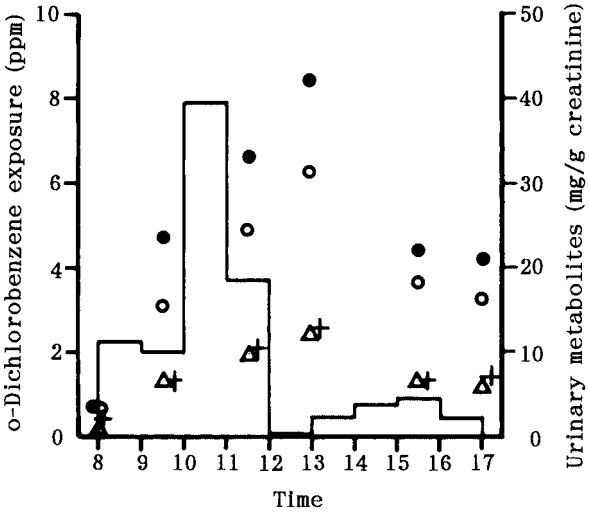

Fig. 5. Concentration of *o*-dichlorobenzene exposure with time and concentrations of urinary metabolites for worker A. ○: 3,4-dichlorocatechol, ●: 4,5-dichlorocatechol, △: 2,3-dichlorophenol, +: 3,4-dichlorophenol. ⎍: 1-hr TWA value for *o*-dichlorobenzene.

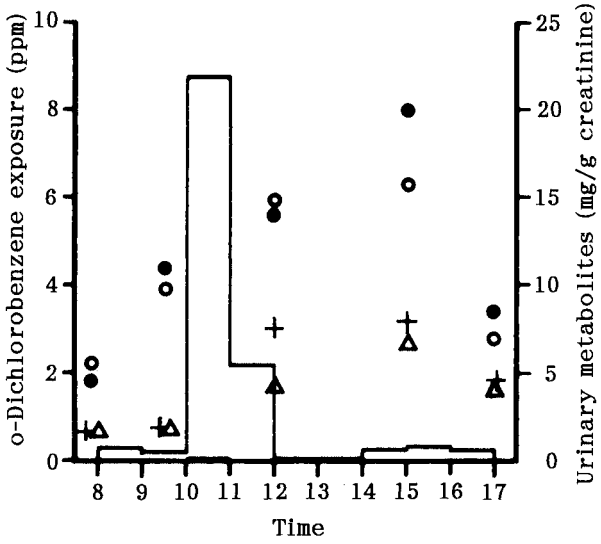

Fig. 6. Concentration of *o*-dichlorobenzene exposure with time and concentrations of urinary metabolites for worker B. ○: 3,4-dichlorocatechol, ●: 4,5-dichlorocatechol, △: 2,3-dichlorophenol, +: 3,4-dichlorophenol. ⎍: 1-hr TWA value for *o*-dichlorobenzene.

4,5-DCC to the 8-hr TWA value was 2.5 times as high as that of 3,4-DCP, the urinary 4,5-DCC level may be more appropriate as a biological indicator of exposure to *o*-DCB. But when our analytical methods are used for routine biological monitoring, quantitative determination of 3,4-DCP may be more efficient than 4,5-DCC, because analysis of one urine sample took less time with Method II (about 25 min) than Method I (about 50 min).

The concentrations of the four urinary metabolites varied with time similarly to each other. For monochlorobenzene, the dihydroxy metabolite, 4-chlorocatechol, is excreted via urine faster than the monohydroxy metabolite, 4-chlorophenol, in humans[13]. According to this tendency, there may be a difference between excretion rates for DCCs and DCPs. In order to clarify this point, a further study with long-term observation after exposure is necessary.

The occupational exposure limit (OEL) for *o*-DCB recommended by the Japan Society for Occupational Health is 25 ppm[14]. The threshold limit value (TLV) for *o*-DCB given by the American Conference of Governmental Industrial Hygienists (ACGIH) is 25 ppm in the United States[15]. It is incorrect to extrapolate the data from this study to estimate the corresponding values for DCC_{en} and DCP_{en}, because the exposure levels in the present study were much lower than the OEL and TLV. Consequently, further examination in higher exposed workers (around OEL level) is necessary to determine such values.

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