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Biological monitoring of workers exposed to dichloromethane, using head-space gas chromatography

Tadashi Sakai[a,*], Yoko Morita[a], Chuji Wakui[b]

[a]*Occupational Poisoning Center, Tokyo Rosai Hospital, 13-21 Omoriminami-4, Ota-ku, Tokyo 143-0013, Japan*
[b]*Keihai Rosai Hospital, Tochigi, Japan*

Abstract

A biological monitoring method for urinary dichloromethane (DCM) has been developed by using head-space gas chromatography with FID detection. The calibration curve is linear in a wide range of DCM levels between 0.01 and 2 mg/l. The recovery rate is almost 100% and within-run coefficients of variation are 2.9–3.7%. A highly significant correlation is found between exposure levels and urinary concentrations of DCM. Determination of urine DCM by this method has many advantages such as sample storage, no need for correction of urine concentration, absence of gender difference and also no confounding effect of glutathione S-transferase T1 polymorphism.

Keywords: Dichloromethane

1. Introduction

Dichloromethane (DCM) is widely used as an industrial solvent in activities such as paint stripping, food processing, and agriculture. The use of DCM is increasing as a replacement solvent for 1,1,1-trichloroethane as its production ceases. The solvent, chlorinated hydrocarbon is known to be toxic to the central nervous system at high exposure levels [1]. Carcinogenicity of DCM has been also reported in mouse lung and liver [2] and there is suspected carcinogenicity in human liver and kidney [3]. As much as 70% of inhaled DCM is absorbed by the pulmonary route. Of the absorbed DCM, 25–34% is excreted as a metabolite, CO, and less than 5% is eliminated unchanged in the expired air [4].

DCM is metabolized in two alternative ways. The substance can be transformed by means of cytoplasmic enzyme, glutathione S-transferase (GST) with glutathione as a co-factor into formaldehyde [4], which is suspected to be a potentially genotoxic intermediate [5]. The GST isoform which is responsible for the transformation is primary GSTT1 (GSTθ1) and may be GSTM1 to a small extent [5]. The oxidative transformation of DCM takes place via the microsomal enzyme, cytochrome P450 2E1 (CYP2E1) [4]. A deletion polymorphism of human GSTT1 results in total loss of activity towards DCM in vitro [3]. Polymorphisms of CYP2E1 in 5′-flanking region base have also been known to alter the metabolic rate of xenobiotic compounds in human [6].

The end products of DCM metabolism in humans are carbon monoxide, carbon dioxide, formic acid, and inorganic chloride. Carbon monoxide binding to Hb (COHb) has been proposed as a biological

*Corresponding author. Tel.: +81-3-3742-7301; fax: +81-3-3743-9082.

E-mail address: opc@msa.biglobe.ne.jp (T. Sakai).

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monitoring index [4]. However, difficulties occur in distinguishing the COHb levels due to the occupational exposure to DCM, from those caused by smoking.

The aim of this study is to examine the possibility of biological monitoring of DCM in urine, using head-space gas chromatography (HS-GC). The confounding effects of physiological and genetic factors on the urinary DCM levels are also investigated.

2. Experimental

2.1. Chemicals

Carbon disulfide was obtained from Wako (Osaka, Japan), and DCM was from Tokyo Kasei Kogyo (Tokyo, Japan).

2.2. Biological materials

Subjects were 95 workers (50 men and 45 women) with occupational exposure to DCM in a printing factory. DCM was used for washing the printing machine and removing ink. Spot urine was collected at the end of the afternoon shift. In addition, spot urine samples were also collected continuously throughout the work shift and thereafter until the following morning from some workers who continued to work after the dinner break.

More than 50 ml of urine were voided into a disposable sample cup made of paper. Air levels of DCM in the room were decreased as low as possible to avoid DCM contamination into urine. Urine was transferred into and filled up a 16-ml glass tube (screw-cupped with a Teflon septum) as soon as possible (within 1 min) after sampling. Pure water instead of urine was used to test that the cup and tube were free of DCM. Thus these materials were not sources of DCM contamination.

The urine samples were kept in a refrigerator until analyses which were mostly carried out on the sampling day or on the following day at the latest. Some of the urine samples were analyzed 4 days after sampling to examine the effect of sample storage on the urinary DCM levels.

2.3. HS-GC determination

Urinary DCM was determined by GC equipped with a flame ionization detector (GC–FID) using head-space gas method. A 1-ml sample of urine sample was put into a 20-ml head-space GC vial (Perkin-Elmer, Norfolk, CT, USA). After the vial was kept at 60°C for 30 min (thermostat time), head-space gas was injected into GC–FID (Model GC-17A, Shimadzu, Kyoto, Japan) connected to an automated head-space air sampler (Model HS-40, Perkin-Elmer), whose pressurized, injection and withdrawal times were set at 3, 0.1, and 0.5 min, respectively. The capillary column used was DB-624 (60 m long, 0.32 mm I.D., 1-μm film thickness; J&W Scientific, Folson, CA, USA). Injector and detector temperature were set at 200 and 250°C, respectively. The column temperature was programmed from 60 to 180°C at 10°C/min. He gas was used as the carrier at a constant pressure of 0.13 MPa.

Before and after the determination of a series of samples (usually more than 30 bottles corresponding to 10–20-h intervals), three bottles of standard (2 mg/l in distilled water) were used for calibration. The levels of both standards were not significantly different.

2.4. Air sampling and GC determination

The time-weighted average (TWA) concentration of DCM vapor in the breathing zone of individual workers was measured by a diffusible sampling method, using 3M personal monitoring badges (#3500 or #3520, 3M, Tokyo, Japan). The badge was attached to the worker's collar during the afternoon work shift. The absorbed DCM was extracted immediately after sampling, with 1.5 ml of carbon disulfide from the carbon felt of the badge. For the determination of DCM, 1 μl of the extract was injected into GC–FID (Model G3000, Hitachi, Tokyo, Japan). The capillary column (TC-WAX, 100 m long, 0.53 mm I.D., 1.0-μm film thickness; GL Science, Tokyo, Japan) was used for the analysis of DCM. Injector and detector temperatures were set at 150 and 180°C, respectively. The column temperature was programmed to be 50°C for the initial 10

min, then increased from 50 to 100°C at 2°C/min. He gas was used as the carrier at a flow-rate of 7 ml/min.

2.5. PCR and polymorphism

Venous blood was collected with EDTA-2K from 42 workers for the extraction of genomic DNA. The study procedure was explained to all workers and their informed consent was obtained. Genomic DNA was isolated from blood samples (0.5 ml) by a DNA extractor kit (Wako, Osaka, Japan). CYP2E1 polymorphism (c1 and c2 alleles) and null type GSTT1 and GSTM1 were determined by the method of Hayashi et al. [7] and Kempkes et al. [8], respectively, using PCR. The DNA amplification was carried out using a Thermal Cycler (Omuni gene, Hybaid, Teddington, Middlesex, UK).

3. Results

3.1. HS-GC detection

HS-GC detection of DCM in urine from workers is shown in Fig. 1. In the urine from workers exposed to the solvent, the DCM peak appears at ~5.75 min and is clearly separated from other components of head-space gas, such as acetone. At the DCM retention time, no or little peak is found in urine from a control subject and distilled water (DW). Increasing amounts of DCM added to urine and DW show a straight line from 0.01 to 2 mg/l (Fig. 2). The both lines are exactly coincident, indicating that the rate of recovery is constant in a wide range of DCM concentrations. Recovery rates of DCM added to control urine (0.6 mg/l) were 99.8±5.3% (n=10). The detection limit was ~0.01 mg/l (S/N=2). When 0.5 and 0.05 mg/l of DCM were added to control urine, within-run coefficients of variation (C.V.) were 2.9 and 3.7% (n=10), respectively.

3.2. Urinary DCM

Fig. 3 shows the DCM concentrations before and after storage of urine for 4 days, when the urine from workers was examined on the sampling day and 4 days later. If the urine is fills more than 95% of the volume of a glass tube (screw-capped and Teflon-sealed) and stored at 4°C, urine can be stored at least for 4 days with little loss of DCM levels.

There is no DCM peak in the control subjects not exposed to the solvent, although trace amount of DCM (less than 0.01–0.02 mg/l) might be detected in some subjects. Urinary DCM increases rapidly

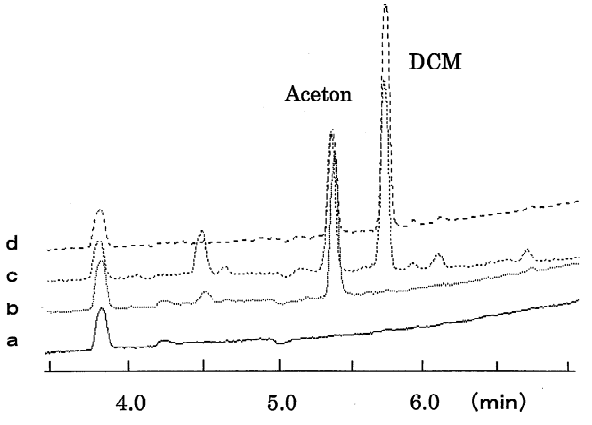

Fig. 1. Head-space gas chromatograms of DCM. (a) Distilled water, (b) control urine, (c) exposed urine, and (d) standard DCM in distilled water (0.5 mg/l).

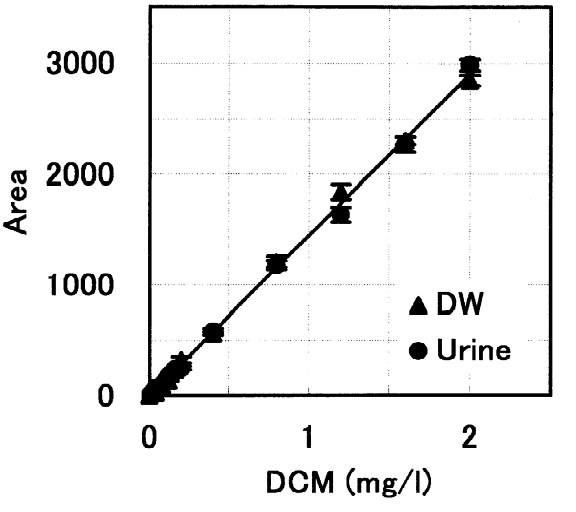

Fig. 2. Calibration curves for DCM in distilled water (DW) and urine from a non-exposed subject. SD is shown in triplicate measurements.

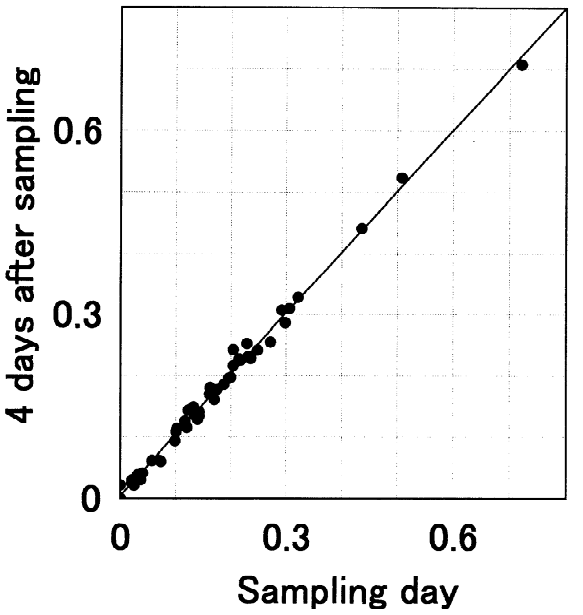

Fig. 3. Relationship of urinary DCM levels (mg/l) determined before and after storage of urine for 4 days at 4°C.

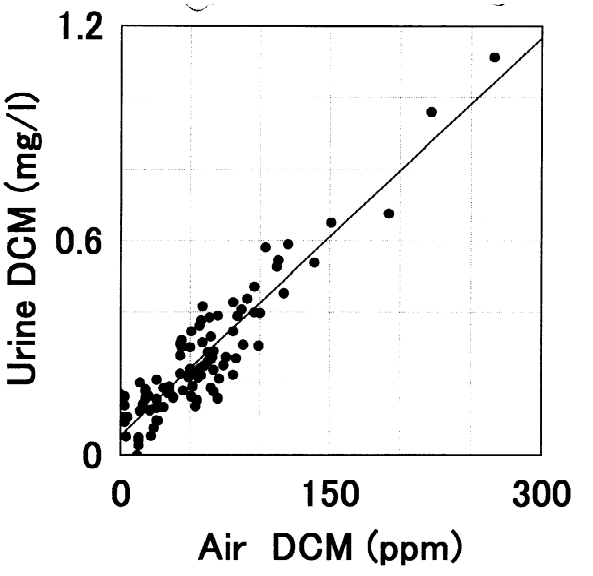

Fig. 5. Relationships between urinary and air DCM levels: $y=0.0037x+0.0545$ ($n=96$, $r=0.924$).

with the start of exposure to DCM in the morning shift and decreases immediately during the lunch and dinner breaks (Fig. 4). The biological half time of DCM excretion is calculated to be 210–400 min from the diminishing curves of three workers after work until the following morning.

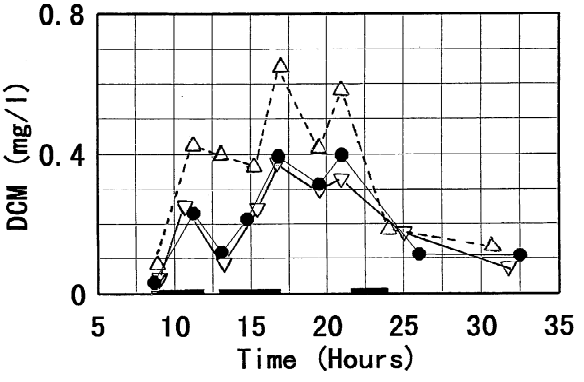

Fig. 4. Time course of urinary DCM levels of three workers during and after exposure to DCM. Bold lines indicate exposure time.

3.3. Correlation of urinary DCM versus exposure

Highly significant correlations are found between exposure levels and urinary concentrations of DCM (Fig. 5 and Table 1). There is no significant difference between gender in the correlation (Fig. 4B,C). Both the slope and intercept are similar in male and female workers. Correlation coefficients of urinary DCM versus personal exposure levels are not improved by correction with specific gravity or creatinine (Table 1).

Even if the workers are divided into two groups with or without GSTT1 gene, correlations of exposure levels versus urinary DCM are not significantly different from each other (Fig. 6). Because the numbers of workers GSTM1 positive are extremely small in comparison with null type GSTM1, differences in the GSTM1 polymorphism could not be clearly determined in the present examination. When the workers are divided into two groups by CYP2E1 genotype, the slope of regression equation of the workers with c2 allele is slightly lower than that of c1 homozygous workers. However, the difference is not significant.

Table 1
Correlation equations of urinary DCM versus air DCM levels

	y	*a*	*b*	*r*
Total	mg/l	0.0037	0.0545	0.924
(*n*=95)	mg/l (SG)	0.0032	0.0725	0.690
	mg/g (Cr)	0.0029	0.0655	0.671
Male	mg/l	0.0038	0.0442	0.887
(*n*=50)	mg/l (SG)	0.0028	0.0652	0.611
	mg/g (Cr)	0.0021	0.0744	0.480
Female	mg/l	0.0036	0.0680	0.941
(*n*=45)	mg/l (SG)	0.0032	0.0980	0.712
	mg/g (Cr)	0.0031	0.0864	0.751

$y = ax + b$ (*x*, ppm), Cr, creatinine; SG, specific gravity.

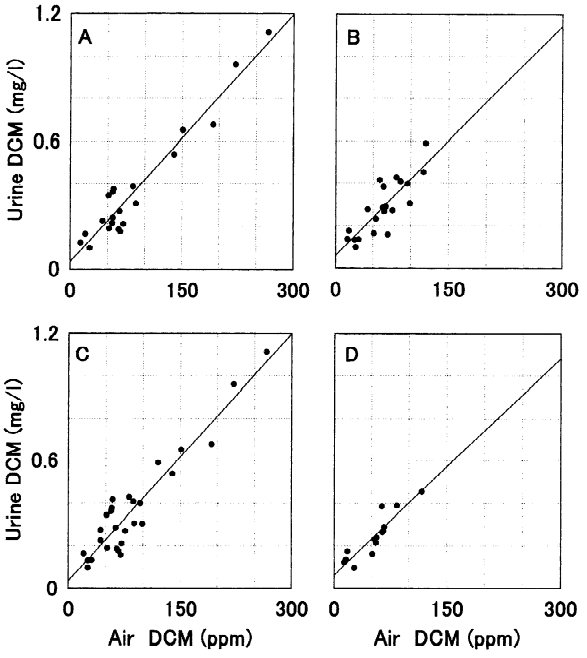

Fig. 6. Relationships between urinary DCM and air DCM levels. (A) Workers with GSTT1: $y=0.0039x+0.0308$ ($n=21$, $r=0.963$), (B) workers without GSTT1: $y=0.0036x+0.0557$ ($n=21$, $r=0.831$), (C) workers without CYP2E1 c2 allele: $y=0.0039x+0.0364$ ($n=28$, $r=0.940$), and (D) workers with CYP2E1 c2 allele: $y=0.0034x+0.0621$ ($n=14$, $r=0.904$).

4. Discussion

The present study has shown that urinary DCM can be simply determined by HS-GC with FID detection. The detection limit of urinary DCM was found to be 0.01 mg/l. The method was sufficiently accurate to detect solvent exposure, since the urinary concentration of DCM corresponding to threshold limit values (TLV) of 50 ppm [9] was estimated to be 0.24 mg/l from the equation in Fig. 5. Although trace or small amounts of DCM were detected in some control subjects, the levels were near or less than the detection limit (0.01 mg/l), which was almost 1/10 of DCM levels of the workers exposed to the TLV level.

In the present study, good correlations between urinary and exposure levels of DCM were obtained (Fig. 5). The correlation coefficient in the total was high at 0.924, which was compatible with that reported by Ukai et al. ($r=0.865$) [10]. They examined 61 workers (46 men and 15 women) exposed to DCM, and reported the correlation equation of $y=0.00372x+0.0173$, between TWA concentration of DCM (*x*, ppm) during a 4-h afternoon shift and DCM in the urine (*y*, mg/l) at the end of the shift. These correlations were nearly same as that found in the present study. Ghittori et al. reported a significant relationship between urinary and air DCM levels [11], although the correlation equation is different from the present one and also from that of Ukai et al. [10].

Correlation of urinary DCM versus personal exposure levels is not only improved but also rather decreased by correction with specific gravity or creatinine (Table 1), indicating that spot urine should be useful for the evaluation of DCM exposure without any correction. In the data of Ukai et al. [10], correlation was not improved by correction. Lessened correlation following correction with specific gravity or creatinine was reported in many kinds of solvent exposure, such as methanol [12], methylethylketone [13], acetone [14], and toluene [15]. In toluene exposure, correlation of hippuric acid versus exposure level was improved, although correction of urinary toluene did not improve the correlation [16]. In general, solvent excretion in urine may not be affected by water balance, although the metabolites may be.

DiVincenzo and Kaplan [4] reported the time course of DCM in blood of volunteers after cessation of exposure, indicating two or more phase of decrease. For 2 h after exposure, DCM in blood decreased very sharply but thereafter the decrease was slower. From this, two or three phases in the decrease of DCM in urine can be presumed. In the present study, however, we could not collect the urine from the workers, because they worked overtime until 10 p.m. It was very difficult to collect urine at midnight from the actual workers. If we have the chance to collect urine consecutively after a work-shift, we would like to try further to obtain more precise data on the many-phase decrease of urinary DCM.

The presence or absence of GSTT1 gene had no effect on urinary excretion of DCM (Fig. 6). The finding confirms that the GST route seems only to become important at doses above the saturation levels of the p-450 route (more than 500 ppm) [1]. The exposure levels of workers in the present study were less than 300 ppm. However workers with CYP2E1 c2 allele showed slightly lower slope of the correlation compared with the c1 homozygous workers, although the difference was not significant. The slightly lower slope of correlation of urinary versus air DCM may be due to the fact that the c2 allele shows a greater transcriptional rate, protein level, and enzyme activity [6], compared to the c1 allele. The differences in excretion of DCM into urine between the c1/c2 polymorphism should be further examined at low exposure levels less than 500 ppm of DCM.

5. Conclusions

For the biological monitoring of workers exposed to DCM, simple determination of urinary DCM has many advantages such as sample storage, no need for the correction of urine concentration, absence of gender difference and also no confounding effect of GSTT1 polymorphism.

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