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Original Article

Biological monitoring of occupational exposure to dichloromethane by means of urinalysis for un-metabolized dichloromethane

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Abstract : The objective of the study is to establish exposure-excretion relationship between dichloromethane (DCM) in air (DCM-A) and in urine (DCM-U) in workplace to confirm a previous report. Male workers in a screen-printing plant participated in the study. Time-weighted average DCM-A was measured by diffusive sampling followed by gas-chromatography (GC), and DCM in end-of-shift urine samples was by head-space GC. The data were subjected to regression and other statistical analyses. In practice, 30 sets of DCM-A and DCM-U values were available. The geometric mean DCM-A was 8.4 ppm and that of DCM-U (as observed) was 41.1 µg/l. The correlation coefficients (0.70–0.85) were statistically significant across the correction for urine density. Thus, the analysis for un-metabolized DCM in end-of-shift urine samples is applicable for biological monitoring of occupational exposure to DCM, in support of and in agreement with the previous report. In conclusion, biological monitoring of occupational DCM exposure is possible by use of analysis for un-metabolized DCM in end-of-shift urine.

Key words: Biological monitoring, Dichloromethane, Exposure-excretion relationship, Methylene chloride, Occupational exposure

Introduction

Dichloromethane (or methylene chloride) (DCM in short; CAS No. 75-09-2) is a highly volatile (boiling point; 39.75°C) but nonflammable chlorinated hydrocarbon solvent. With regard to its toxicity, the depressive effect on the central nervous system has been well documented[1–3]. In addition, cases of occupational bile duct cancer[4, 5] were detected among printers in Japan, who were exposed to 1,2-dichloropropane (1,2-DCP) at high concentrations[6–8]. Because the victims were exposed also to DCM at high levels[6–8], the causative effects of DCM in addition to that of 1,2-DCP was suspected[6–8].

In 2017, International Agency for Research on Cancer[9] moved DCM from 2B to 2A in the carcinogenicity classification; in short, human studies (cohort and case-control studies) had limitations (e.g., small in study size or co-exposure to other solvents) but animal studies were conclusive (e.g., significant increase in hepatocellular ademona/carcinoma). The change was followed by Japan Society for Occupational Health[10]. In succeeding years, association of various diseases with DCM exposure was reported. For example, association of hypopharyngeal

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Table 1. Exposure parameters

Paramter	Age (yr)	DCM-A[a] (ppm)	DCM-U[b] as			Creatinine (g/l)	Specific gravity
			Observed (μg/l)	Correlated for CR[c] (μg/g)	Correlated for SG[d] (μg/l)		
n	30	30	30	30	30	30	30
Min	19	1.9	18	10	12	0.510	1.009
Max	60	39.9	148	99	85	3.132	1.031
GM e	30.5[g]	8.4	41.1	27.2	28.3	1.64[g]	1.024[g]
GSD f	10.4[h]	1.8	1.6	1.8	1.6	0.67[h]	0.18[h]

[a]8-hour average DCM in air, [b]Level in the end of shift urine, [c]Corrected for creatinine concentration (g/l), [d]Corrected for a specific gravity of 1.016, [e]Geometric mean, [f]Geometric standard deviation, [g]Arithmetic mean, [h]Arithmetic standard deviation.

cancer with occupational DCM exposure was reported for men[11], although not for women[12]. Industrial DCM release may be a risk factor of childhood germ cell tumors, teratomas and possibly acute myelogenous leukemia[13]. DCM exposure as a risk factor of amyotrophic lateral sclerosis was also reported[14]. Lack of association was reported between DCM exposure and kidney cancer[15].

As DCM is a skin-penetrating solvent[16, 17], air monitoring alone is apparently insufficient to detect exposures through various routes. Therefore, establishment and confirmation of biological monitoring are an up-to-date issue in occupational and public health. It should be noted that the best practice in use of protective gloves (to prevent dermal absorption) is not always expectable. For example, some workers prefer to work without bulky protective gloves, depending on the work type. In the present report, a successful validation of old-time report by Ukai *et al.*[18] will be presented.

Materials and Methods

The workplace surveyed was a screen-printing plant with male workers who used DCM for cleaning of used printing rolls to remove remaining ink and other materials. 1,2-DCP was also employed to remove stains from running rolls, but DCM and 1,2-DCP were never used as a mixture. The working conditions and survey methods were as previously described[19]. In short, the workers served 8 h daily with protective gloves but no respiration masks. Personal 8-h air monitoring was conducted by diffusive sampling[19]. End-of-shift urine samples were collected with due care not to allow the DCM to escape from urine samples[20]. A method has been developed for rapid transfer of each urine sample to a closed vessel (i.e. 5-ml vacuum tube originally developed for blood sampling)[21]. It is important in the practice of good quality control that the transfer of the urine sample from a vacuum tube to a head-space vial should be carried out one-by-one, and never open more than one tube at one time. The transfer should be conducted quickly but steadily. Analysis of DCM in exposed activated carbon cloth was by FID-GC[19]. DCM in urine samples was analyzed by head-space GC[19].

The limits of determination were 0.1 ppm and 1 μg/l (as observed) for DCM in air and DCM in urine, respectively. In practice, 30 cases were available (Table 1). Regression analyses followed by comparison between two regression lines were employed for statistical evaluation after Ichihara[22].

Each of the participating workers submitted his informed consent. The study protocol was approved by the Ethics Committee of Occupational Health Service Center, Japan Occupational Safety and Health Association, Tokyo, Japan. The Board considered that the study met with the exemption criteria[23].

Results

The geometric mean (GM) DCM-A was 8.4 ppm and DCM-A distributed in a wide range of 2 to 40 ppm. DCM-U (as observed) distributed in a range of 18 to 148 μg/l with a GM of 41 μg/l. The maximum values for both DCM-A and DCM-U were less than the occupational exposure limit of 50 ppm and 0.2 mg/l (=200 μg/l)[10], respectively.

After correction of DCM-U for none (i.e., as observed), for creatinine concentration or for a specific gravity of 1.016, DCM-U was subjected to regression analysis with DCM-A, taking DCM-A as an independent variable and DCM-U as a dependent variable. The correlations are depicted in Fig. 1. The regression equation (n=30) was

(A; as observed)

DCM-U (μg/l) = 15.4 + 3.0 × DCM-A (ppm), r=0.848, $p<0.01$,

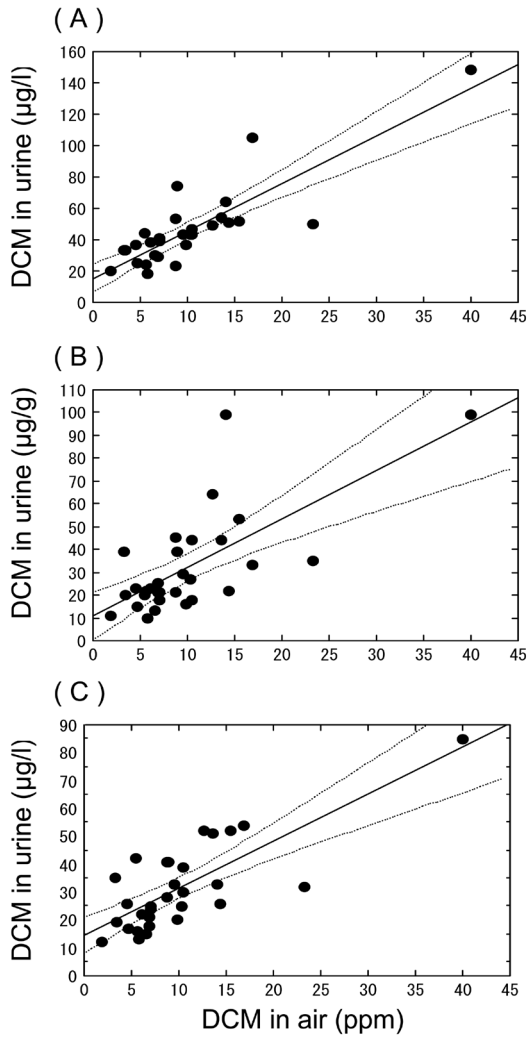

Fig. 1. Linear regression between dichloromethane in air (ppm) and in urine.

(A) DCM-U as observed (unit: µg/l), (B) DCM-U as adjusted for creatinine concentration (unit: µg/g creatinine), (C) DVM-U as adjusted for a specific gravity of 1.016 (unit: µg/l).

The lines in the middle are calculated regression lines, and the curves on both sides of the lines show 95% confidence ranges. Each dot represents one case studied (n=30).

(B; after creatinine correction)

DCM-U (µg/g)=10.9 + 2.1 × DCM-A (ppm), r=0.697, $p<0.01$), and

(C; after correction for a specific gravity of 1.016),

DCM-U (µg/l)=14.6 + 1.7 × DCM-A (ppm), r=0.775, $p<0.01$.

Thus, it was clear that DCM-U (either in µg/l or µg/g creatinine) correlates significantly with DCM-A, (in ppm). The observation suggests that DCM-U can be quantitatively estimated from DCM-A.

Discussion

Perusal of Fig. 1 (A), (B) and (C) suggests that the overall correlation between CDM-A and CDM-U was strongly influenced by one case exposed at 40 ppm irrespective of urine density correction. To examine this possibility, the 40 ppm exposure case was tentatively deleted and correlation analysis was conducted with remaining 29 cases. The correlation coefficients insignificantly dropped to 0.49–0.65 ($p<0.01$), but the changes in intercepts and slopes were all insignificant ($p>0.05$). Thus, the effect considered should be small if present. No further consideration on this possibility was considered to be necessary.

The present analyses made it clear that biological monitoring of occupational exposure to DCM is possible by means of urinalysis for un-metabolized DCM.

Ukai *et al.*[18] previously reported a regression line of Y=7.7 + 3.22X (r=0.91, $p<0.01$), where X was 8-h TWA DCM in ppm, and Y was DCM in µg/l (as observed) in end-of-shift urine. The present observation (Table 1) gives a slightly smaller slope (3.03 µg/l/ppm) and a larger intercept (15.4 µg/l). The comparison of the estimates at DCM-A=40 ppm (the highest exposure concentration observed in the present study) shows that the estimates after Ukai *et al.*[18] is 137 µg/l (95% range: 124–151 µg/l), whereas the corresponding values by the present observation is 136 (113–160) µg/l. Taking the variation range into consideration, the two surveys give essentially the same results.

Thus, the results of analyses conducted in two analytical laboratories (one in Osaka Occupational Health Service Center, Japan Industrial Safety and Health Association where once Kawai served and the other in Kyoto Industrial Health Association) agreed very well to each other. It was considered that the analytical method employed are valid and the equations given above in the Results section is applicable in present day surveys.

Conclusions

Analysis for un-metabolized DCM in end-of-shift urine is applicable for biological monitoring of occupational exposure to DCM. The observation by Ukai *et al.*[18] is reconfirmed and validated.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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