水	r =0.99992	n＝8	水	r =0.99997	n＝6
	Y=2.18e+3X-780			Y=2e+3X-78.3	
尿添加	r =0.99985	n＝8	尿添加	r =0.99819	n＝6
	Y=2.04e+3X-1.6e+3			Y=1.77e+3X-657	

表１．尿中ヒ素代謝物濃度（N=66, μg/L）

ヒ素化合物	平均 ± 標準偏差	範囲
無機ヒ素（５価）	0.5 ± 1.1	<0.1 - 46.6
無機ヒ素（３価）	5.3 ± 11.0	<0.1 - 49.6
メチルアルソン酸	3.1 ± 4.8	<0.1 - -28.5
ジメチルアルシン酸	64.4 ± 46.6	5.4 - 198
アルセノベタイン	1193 ± 102	11.0 - 351
ジフェニルアルシン酸	<0.3	<0.3
総ヒ素量	217 ± 147	24.9 - 707

D. 考察

化学兵器の処理作業で最も問題となる曝露は、ヒ素系のジフェニルアルシン化合物である。この化学物質暴露による生物学的モニタリング法としては、今回の尿試料による陽イオンカラムを用いた HPLC-IC-MS 分析法により、代謝物である DPAA の検出感度も良く、検量線の直線性も良く、安定した高感度分析が可能となった。

一方、ルイサイトなどの曝露においては、尿中無機ヒ素が対象物質となるので、ヒ素化合物の分離分析が必要となる。本研究において、無機ヒ素から DPAA までのヒ素化合物の一斉分析が可能であることが示されたので、化学兵器処理作業者の生物学的モニタリング法として、本方法が適切であることが明らかになった。

この方法により、作業前の尿中濃度測定を実施した結果、DPAA は検出されなかった。この結果は、通常の環境においてはジフェニルアルシン化合物が存在しないことを示しており、予想通りであった。しかしながら、尿中ヒ素化合物濃度は表１に明らかなように総ヒ素量としては高濃度であった。ヒ素化合物のうち、AsBe が総ヒ素量のほぼ 2/3 を占めたのは、彼らが、海岸

部に居住するため、海産物摂取が多いからだと推定された。また、メチル化ヒ素のうちDMAが総ヒ素量の約1/3を占めていた。無機ヒ素は体内でメチル化され、その代謝物としてはモノメチルアルソン酸(MMA)とDMAが尿中に排泄される。今回、高濃度のDMAが尿中から検出されたが、他の無機砒素およびMMAの合計量は総ヒ素量の約5%とDMAよりかなり少ないので、検出されたDMAは無機ヒ素由来ではなく海産物由来だと推定される。

米国ACGIHは無機ヒ素曝露の許容濃度としてTLV-TWA 0.01mg/m^3を勧告し、生物学的許容値(BEI)に無機ヒ素とメチル化ヒ素の合計濃度として35μg As /Lを設定している。また、ドイツ(DFG)は管理濃度0.01mg/m^3に対応する尿中ヒ素濃度(無機ヒ素+メチル化ヒ素)として50μg As /Lを設定している。日本人においては、本研究でも明らかなように海産物摂取によるヒ素濃度の上昇が顕著で、尿中DMA濃度も高いことが知られている。今回の結果においても、66名のDMA平均値はこれだけでいずれの国の勧告値も超えてしまっている。

以上のごとく、日本人においては、海産物摂取と関係するヒ素化合物が高濃度に検出され、諸外国で使われている無機ヒ素とメチル化ヒ素の合計濃度を日本人におけるヒ素曝露の指標として用いると、DMAの影響が大きいことから、無機ヒ素曝露の指標にDMAを入れることは、日本人においては適切ではないと考えられた。作業後の尿中濃度については、まだ採尿が終わっておらず、検討は今後の課題である。

E. 結論

1) ヒ素系化学兵器曝露評価には、尿を用いた生物学的モニタリングをする必要がある。
2) 測定対象物質としてはトリクロロアルシン、ルイサイトの曝露については加水分解産物である尿中無機ヒ素、ジフェニルクロロアルシン、ジフェニルシアノアルシンの曝露についてはそれらの分解物である尿中DPAAとした。
3) 陽イオン交換カラムを用いたHPLC-ICP-MS分析により、尿中のDPAAと無機ヒ素の分離分析を30分で実施できた。
4) DPAAの検出下限は0.3μg /L、検量線は0-100μg /Lにおいて直線性が得られ、再現性もCV=4%と良好であった。
5) 尿にDPAAを20 μg/L濃度で添加したときの回収率は92.9±6.2%(n=20)であった。
6) 日本人では、非曝露者においても尿中総ヒ素は高濃度であった。
7) 無機ヒ素曝露の指標に、DMAを入れることは問題がある。

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G. 知的所有権の取得状況

なし

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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.

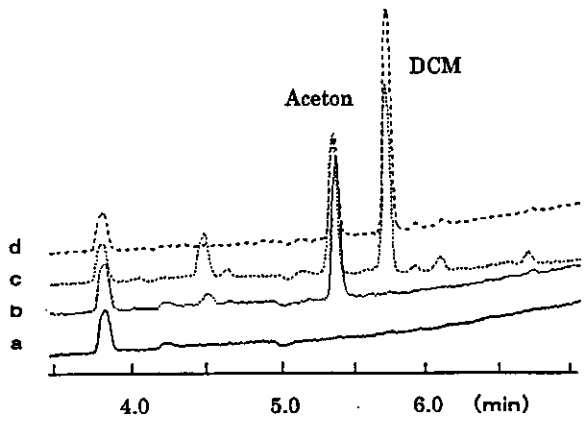

Fig. 1. Head-space gas chromatograms of DCM. (a) Distilled water, (b) control urine, (c) exposed urine, and (d) standard DCM in distilled water (0.5 mg/l).

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

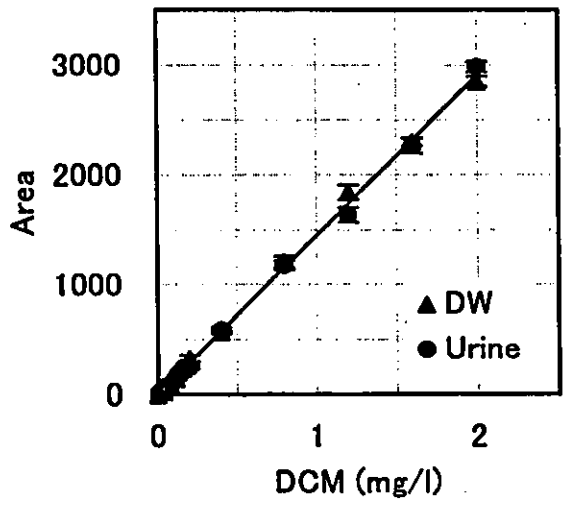

Fig. 2. Calibration curves for DCM in distilled water (DW) and urine from a non-exposed subject. SD is shown in triplicate measurements.

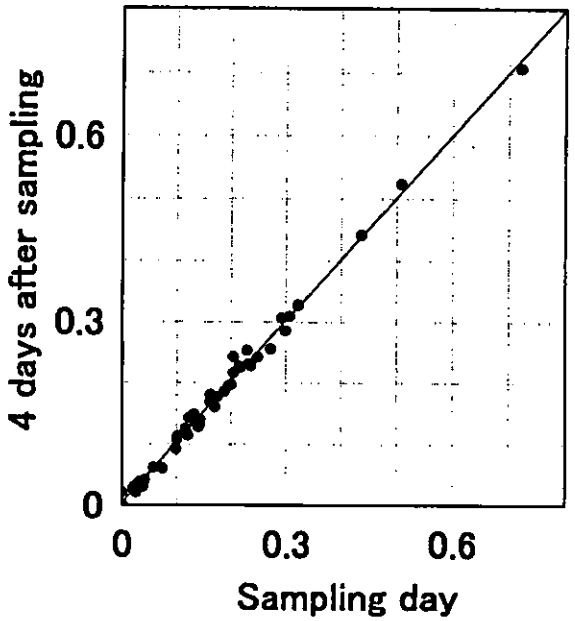

Fig. 3. Relationship of urinary DCM levels (mg/l) determined before and after storage of urine for 4 days at 4°C.

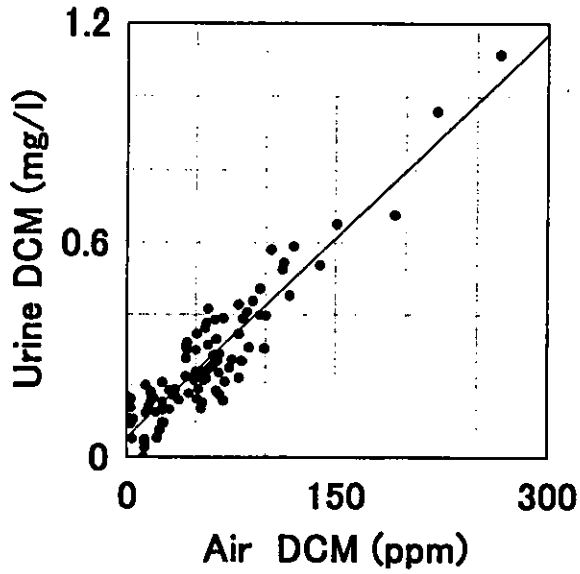

Fig. 5. Relationships between urinary and air DCM levels: $y=0.0037x+0.0545$ ($n=96$, $r=0.924$).

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.

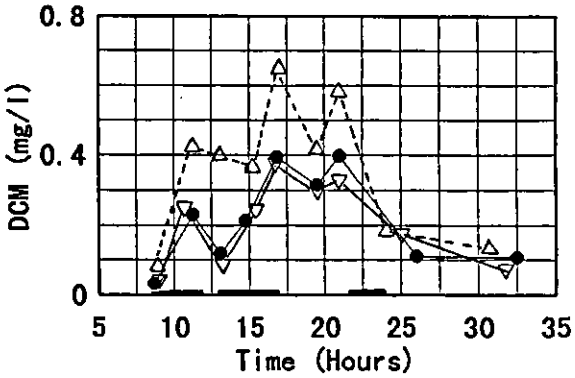

Fig. 4. Time course of urinary DCM levels of three workers during and after exposure to DCM. Bold lines indicate exposure time.

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.

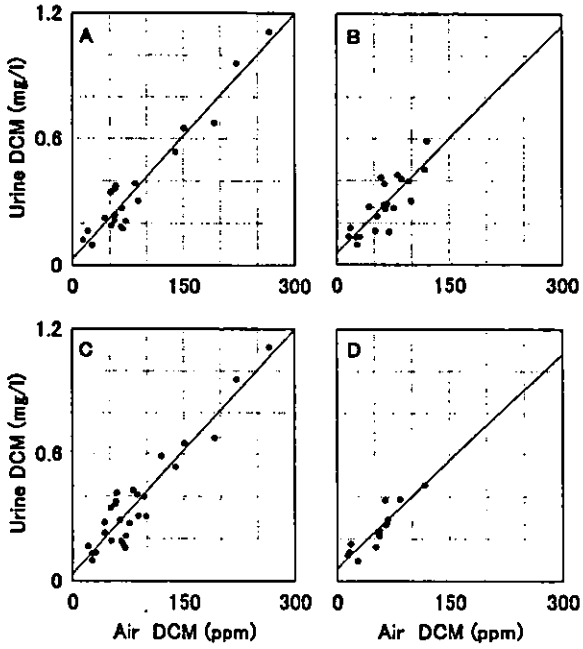

Fig. 6. Relationships between urinary DCM and air DCM levels. (A) Workers with GSTT1: $y=0.0039x+0.0308$ ($n=21$, $r=0.963$), (B) workers without GSTT1: $y=0.0036x+0.0557$ ($n=21$, $r=0.831$), (C) workers without CYP2E1 c2 allele: $y=0.0039x+0.0364$ ($n=28$, $r=0.940$), and (D) workers with CYP2E1 c2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

DETERMINATION OF LEAD IN PLASMA, WHOLE BLOOD, AND URINE BY ICP-MS AND THE RELATIONSHIPS AMONG THE THREE EXPOSURE INDICES

Tadashi SAKAI, Yoko MORITA and Takaharu ARAKI

Occupational Poisoning Center, Tokyo Rosai Hospital

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Abstract

The cbjectives of this study were to develop the simple method of datermining lead in plasma (PbP), whole blood (PbB), and urine (PbU) by inductively coupled plasma mass spectrometry (ICP-MS), and to examine the relationships among the three indices of lead exposure. Venous blood treated with heparin and spot urine was collected from 69 male lead workers. For ICP-MS analyses, plasma, whole blood, and urine were diluted 5, 50, and 80-fold, with nitric acid solution, respectively. PbP, PbB, PbU were also detarmined by the method of atomic absorption spectrometry (AAS). PbB and PbU determined by ICP-MS were close to those by AAS, however, PbP levels by AAS were more than 2-times higher than those by ICP-MS. PbP and PbU levels logarithmically increased with elevating PbB. A linear relationship was found between PbP and PbU. PbP, PbB and PbU were simply measured by ICP-MS, and the logarithmic or linear relationships were found among those indices.

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—Key words—
Lead, Plasma, ICP-MS

Introduction

In blood, more than 98% of the lead are found in blood cells[1)2)]. Plasma lead (PbP) has an important role in lead metabolism, where it is the active center of the body lead pool, although the concentration is very low levels. PbP is equilibrates with the extra-cellular pool and is directly involved in all the movements of lead among the different biological compartments[3)]. Thus, lead in plasma circulates in the body, affects the body lead burden and causes the toxicity of lead in some soft tissuies, such as bone marrow, kidney brain and so on.

The levels of PbP are sharply elevated with a sudden intake or acute exposure to lead and rapidly diminished by time elapse from it[3)], indicating that PbP is an index for very recnt exposure[4)]. The chelatable lead excreted in urine (MPbU) is considered to be an excellent measue of potentially toxic fraction of the body lead burden[5)], and usually determination in urine collected for 24 hs after calcium disodium ethylenediamine tetraacetic acid (Ca EDTA) administration[6)]. Recently, Sakai et al[7)]. have report that PbP concentration at 2 hs after the start of CaEDTA injection (MPbP) is well correlated with the amount of lead excreted in urine for 24 hs thereafter, and is a useful measure for the chelatable lead.

Thus, PbP as well as MPbU are very impotrant indices of lead as exposure, distribution, and health risk. However, the concentration of lead in plasma or serum is not routinely measured, so far. One of the main reasons is the very low conecentration of PbP, which is difficult to determine by atomic absorption spectrometry (AAS). In the AAS techniques, it needs troublesome procedures of chelation and extraction[1)], which might result in contamination of samples with exogenous lead.

Recently, a highly sensitive instrument, inductively coupled plasma mass spectrometry (ICP-MS), has been introduced for the measurement of lead in plasma or in serum[2)8)-11)]. Lead in whole blood (PbB) and in urine (PbU) can be also determined by ICP-MS more exactly than AAS[12)9)12)~14)].

The aim of the present study is to establish the ICP-MS method for the determination of PbP, PbB, and PbU

in lead workers. We have also investigated the relationships among the three indices of lead exposure, using ICP-MS.

Materials and Methods

The subjects were 69 male lead workers (25-61 years old, mean 43 years). They were employed in a battery smelter and a glass factory. Venous blood (n=69) and spot urine (n=49) were collected during their physical examination. Urine was collected in acid washed polypropylene cups. Venous blood was drawn from the cubital vein into an evacuated and heparinised 5 ml tube (Terumo, Tokyo, Japan). Plasma was separated by centrifugation (10 min, 1,200 g) within 30 min after the sampling and transferred into acid washed polypropylene tubes. The samples of blood, plasma, and urine were stored at −20°C. The analysis was carried out within 3 months after sampling.

Standard solutions containing a single element (1,000 mg/l) of lead and bismuth were purchased from Wako Pure Chemicals (Osaka, Japan). Ultra pure nitric acid (Tama Pure AA-10) was purchased from Tama Chemicals (Kawasaki, Japan). Distilled pure water (DW) was prepared by filtration of distilled water through a Millipore-Q system (Millipore Japan, Tokyo, Japan).

For preparation of sample of solutions for ICP-MS determination, whole blood (30 µl) was hemolyzed with 270µl of DW, then well mixed with 1.2 ml of nitric acid (0.15 N). Plasma (300 µl) was well mixd with 1.2 ml of nitric acid (0.15 N). Plasma (300 ml) was well mixed with 1.2ml of nitric acid (0.15 N). Thirty microliter of bismuth nitrate (500 µg/l) was added to each sample as an internal standard. In the same way, 30 µl of urite was well mixed with 2.4 ml of the nitric acid and 50 µl of the bismuth solution was added to each sample.

ICP-MS (Model PMS-2,000, Yokogawa Analytical Systems, Tokyo, Japan) was used for the analysis of lead in the sample solutions. The operating conditions were as follows : RF power 1.2 kW, plasma gas 14 l/min, auxiliary gas 1 l/min, carrier gas 0.98 l/min, and sample uptake rate 0.4 ml/min, respectively. The selected isotopes ^{208}Pb and ^{209}Bi were monitored at three points per peak. The dwell time was 10 ms/point for both Pb and Bi. They were scanned 50 times in a determination. One sample was determined 3 times and total analytical time was 9.6 second. The average of five replications was used for calculations. The instrument was calibrated against spiked plasma (+10 and +20 µg/l), whole blood (+500 and +1,000 µg/l) and urine (+100 and +200 µg/l) samples.

In all 69 workers, the lwvels of PbP were determined by the method of ICP-MS. For the comparison, 19 out of 69 lead workers were determined by the method of DeSilva[1], using graphite furnace AAS (Model Z-8,000, Hitachi, Tokyo, Japan). For the comparison, PbB was also measured by both ICP-MS and graphite furnace AAS in 53 out of 69. PbU was determined by ICP-MS in 49 lead workers. In 15 workers of them, PbU was analyzed by the method of Ushio et al[15]. using flame AAS (Model Z-8,000, Hitachi, Tokyo, Japan) for the comparison. For the external quality control of PbB determination, our laboratory was involved in the Quality Control Program conducted by the National Federation of Industrial Health Organizations, with the support of Ministry of Labor, Japan. PbU was corrected for creatinine concentrations. Creatine was determined by the method of Jaffe with the "Creatinine Determination Kit" of Wako Pure Chemicals (Osaka, Japan).

Results

Fig. 1 shows the calibration curves of PbP, PbB and PbU in ICP-MS analyses, indicating ICP-MS has a wide dynamic range in every determination. In the method of ICP-MS, the recovery rate of PbP was 105.9 ± 4.3%, when 10 µg/l of Pb was added to 20 plasma samples (PbP range : 0.34-17.18 µg/l). The mean recovery rate of PbB was 100.0 ± 1.2% when 500 µg/l of Pb was added to 12 blood samples (PbB range : 42~679 µg/l). In the same way, the recovery of PbU was 101.5 ± 2.2% when 200 µg/l of Pb was added to 8 urine samples (PbU range : 7.3~200.8 µg/l, creatinine concentration range : 48.9~193.9 mg/dl).

Table 1 indicates the comparison of mean and range of PbP, PbB and PbU determined by ICP-MS and AAS. Fig. 2 shows the comparison of PbB, PbP, and PbU by the two methods, ICP-MS and AAS. PbB values determined by ICP-MS are well correlated with those by AAS. The PbB values of two methods are almost the same (Table 1).

Although, PbU levels determined by ICP-MS are slightly high in comparison with those by AAS, the correlation coefficient of PbU levels by both the methods is also high (r=0.934). This indicates that PbU measurements by both the methods can be comparable. The mean levels of PbP measured by AAS are more than 2-times higher than those

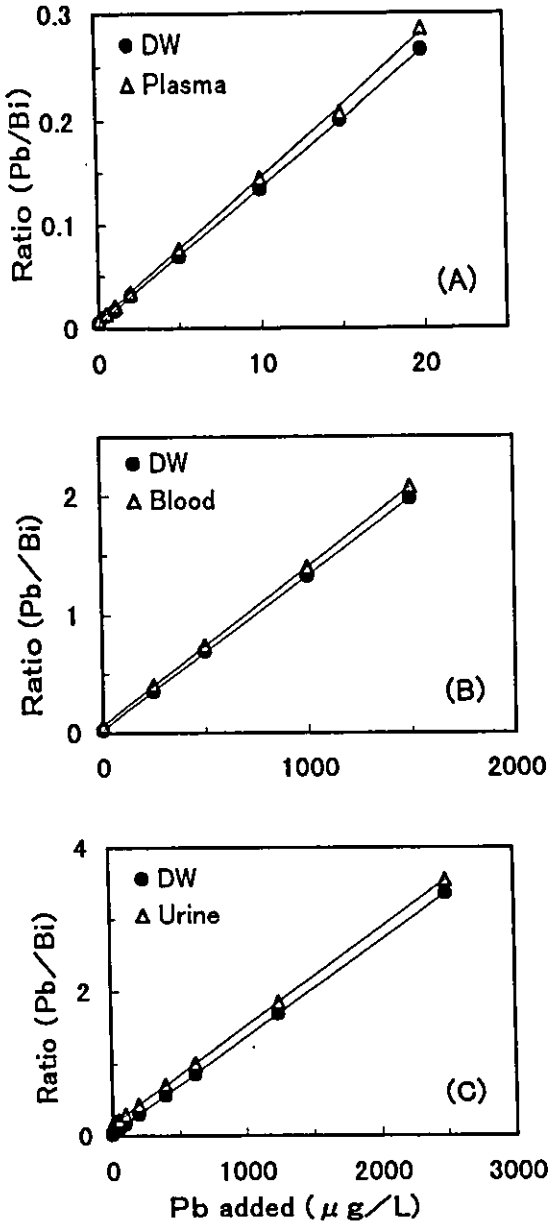

Fig. 1 Calibration curves of PbP (A), PbB (B), and PbU (C). Lead was added to DW or sample.
(A) DW : y=0.013x+0.004 (r=0.999). Plasma : y=0.014x+0.007 (r=0.999).
The mean recovery rate of PbP was 105.8%, when 0.5 to 20 μg/l of lead were added to plasma sample (Original PbP level : 0.57 μg/l).
(B) DW : y=0.001x+0.016 (r=1.00). Blood : y=0.001x+0.061 (r=1.00).
The mean recovery rate of PbB was 100.8%, when 250 to 1,500 μg/l of lead were added to blood sample (Orignal PbB level : 42 μg/l).
(C) DW : y=0.001x+0.025 (r=1.00). Urine : y=0.001x+0.154 (r=1.00).
The mean recovery rate of PbB was 100.0%, when 25 to 2,500 μg/l of lead were added to urine sample (Original PbU levels : 102.9 μg/l, Creatinine level : 100.2 mg/dl).

by ICP-MS (Table 1). Each PbP level measured by AAS was also apparantly higher (max 9 times) than that by ICP-MS. The difference in both the methods is more evident especially in low PbP levels.

Fig. 3 shows the relationships among PbB, PbP and PbU, which were determined by ICP-MS (Table 1, first raw). Fig. 3 (A) shows the relationship between PbB and PbU (n=53). PbP levels logarithmically increase with elevating PbB levels. The correlation coefficient was as high as 0.933. Fig. 3 (B) shows the relationship between PbB and PbU (n=49). PbU levels are also logarithmically increased with elevating PbB levels. The correlation coefficient (r=0.817) was slightly higher than that calculated in a linear correlation mode (r=0.765). although the difference was not significant. Figure 3 (C) shows the relationship between PbU and PbP (n=49). A linear relationship (r=0.657) is found between PbP and PbU. The correlation coefficient was almost the same as that calculated in a

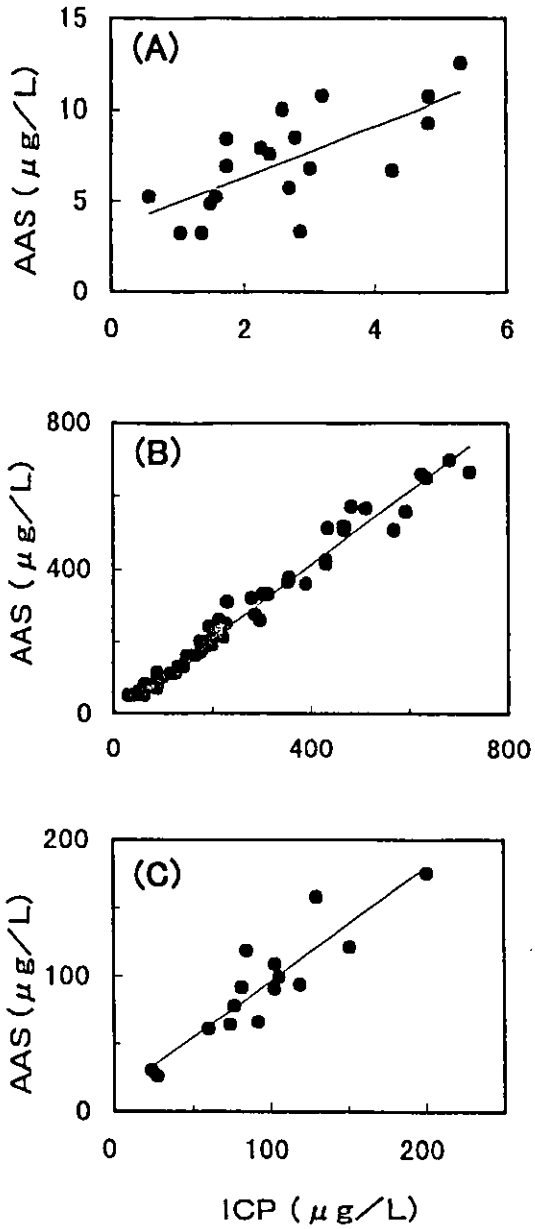

Fig. 2 Comparison of lead determinations by ICP-MS (x) and AAS (y) methods.
(A) PbP : y=1.41x+3.4 (r=0.699, n=19)
(B) PbB : y=1.006x+7.89 (r=0.987, n=53)
(C) PbU : y=0.836x+12.5 (r=0.934, n=15)

logarithmic mode (r=0.656).

Discussion

In the present study, three kinds of indices of lead exposure (PbP, PbB, and PbU) could be simply determined by ICP-MS. For the analysis of plasma, whole blood, and urine, samples were diluted 5, 50 and 80-fold, with nitric acid solution, respectively. Each value of exposure indices was compared by two methods, ICP-MS and AAS (Fig. 2, Table 1). One of the advantages for ICP-MS determination was of a wide range of linearity in the calibration curves (Fig. 1) as compared with AAS, where dynamic range was narrow. Another advantage for ICP-MS determination was of simple procedure and also high sensitivity, which enabled us to determine very low levels of PbP, without complicated and time consuming pretreatment.

Previously we have to determine PbP by graphite furnace AAS after solvent extraction of plasma lead chelat-

Table 1 Comparison of the methods of ICP-MS and AAS for measurement of PbP, PbB, and PbU

Kind of indices	N	Analytical methods	
		ICP-MS	AAS
PbP (μg/l)	19	2.67 ± 1.34 (0.58—5.32)	7.19 ± 2.71 (3.2—12.5)
	69	*1.87 ± 2.26 (0.25—17.18)*	
PbB (μg/l)	53	270 ± 183 (32—724)	276.4 ± 186 (50—698)
PbU (μg/l)	15	95.7 ± 44.9 (24.2—200.8)	92.5 ± 41.4 (26.1—175.9)
	49	*47.4 ± 42.6 (7.3—200.8)*	

Mean ± SD (range)

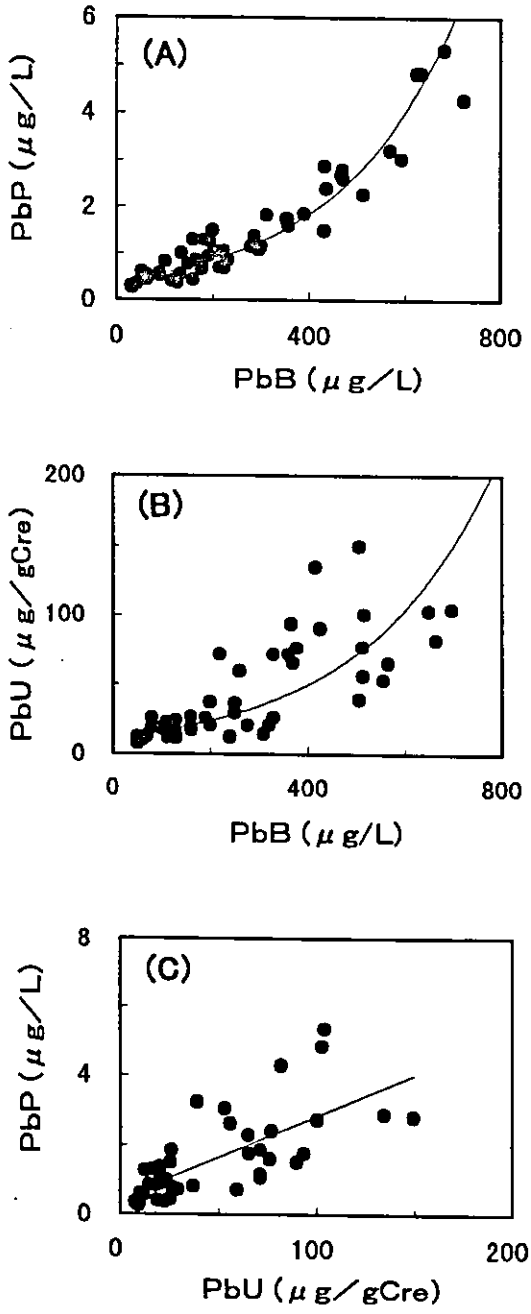

Fig. 3. Relationships among three exposure indices determined by ICP-MS.
(A) PbP (y) vs. PbB (x) : y=0.392e (r=0.933, n=53).
(B) PbU (y) vs. PbB (x) : y=11.78e 0.0035x (r=0.817, n=49).
(C) PbP (y) vs. PbU (x) : y=0.219x+0.481 (r=0.657, n=49).

ed with ammounium pyrrolidinedithiocarbamate (APDC)[4]. The procedure included so many steps and chemicals which mighe cause the contamination of samples with exogenous lead. Acutually PbP levels measured by AAS were much higher than those by ICP-MS. It might be attributable to the contamination of lead during the chelating and extraction procedure befor introducing the sample into AAS. The differences in PbP levels between two methods were especially large at low PbP levels, where the lead contamination might be more obviously observed.

On the contrary, the PbB levels by ICP-MS were almost the same as those by AAS (Fig. 2). The fact indicated that both the methods were useful for the routine analysis. The values of PbU by ICP-MS were also identical to those by AAS. However, it should be further confirmed the exact relationship between PbU levels by two analytical methods in a larger numbers of samples. Thus, ICP-MS method will be used widely in future because the analytical procedure is simpler than by AAS.

Using ICP-MS, Schütz et al[2]. reported the ratio of PbP/PbB increased with increasing PbB, in other words, there was a logarithmic relationship between PbB and PbP. Our result (Fig. 3) also showed that PbP levels logarithmically increased with elevating PbB. Based on the correlation equation in the present study, the PbP level corresponding to PbB levels of 400 μg/l was calculated to be about 1.9 μg/l. The PbP level corresponding to PbB of 400 μg/l was close to that in the report by Schütz et al[2]. from whose data it was calcuated to be about 2.2 μg/l.

PbP is considered to be "biologically active lead" and it is in the equilibrium with the extra-cellular pool of every tissue or organ[3]. The concentration of PbP and serum lead (PbS) has been shown to be very low, usually less than 1% of that PbB, but those percentage are increased with rising lead level[16]. Moreover, Bergdahl et al[17] , who were in the same study group of Schütz, further studied the relationship among PbP, PbB, and bone lead, and reported again positive correlation not only between the ratio of PbP/PbB and bone lead, but also between that and PbB. By the correlation equation[10], the PbP level corresponding to PbB level of 400 μg/l is calculated to be about 6.7 μg/l. The value is 2~3 times higher than those by the data in the present study (1.9 μg/l) and by Schütz et al (2.2 μg/l)[2]. Thus, the data in the present study supported the Schütz, although the bone lead was not determined in the present study.

Since 90% or more of lead in an adults is found in bone and the biological half time of lead in bone is very long (more than several years), it has been considered that bone lead is an important index for internal exposure to lead, but not for external lead exposure[10]. PbP is an index for very recent external exposure[4], might be more directly related to PbU rather than PbB. To examine the relationship between external and internal exposure, it is necessary to examine the relationship among PbP, PbB, and also PbU other than bone lead. In the present study, we demonstrated the relationships among the three exposure indices which could be determined by ICP-MS.

Hirata et al.[18] reported the relationship among PbP, PbB, and PbU using AAS. They found a linear relationship between PbP and PbU and the present data agreed with them. The PbP level corresponding to 100 μg/l of PbU was reportedly be 3.8 μg/l in their workers, although the level was 2.7 μg/g Cre in our present date. They also reported a liner correlation between PbB and PbP or PbU. In the present study, however, a logarithmic relationship was found between PbB and PbP or PbU. Logarithmic relationship between PbB amd PbP are also reported by Schütz et al.[2] and Bergdahl et al.[16]. Furthermore, the PbP level (4.5 μg/l) corresponding to the PbB level of 400 μg/l in the study of Hirata et al.[18] is 2-times higher than those of Schütz et al. (2.2 μg/l)[2], Bergdahl et al (2.1 μg/l)[16] and ours (1.9 μg/l). In the same way, the PbU level (99 μg/l) at the PbB levels of 400 μg/l in their study[18] was also 2-times higher than those in ours (48 μg/gCre, Fig. 3). These disagreements might be due to the differences in the method of determination (AAS) and in the levels of lead exposure (PbB : 308-1,020 μg/l) in their study.

The relationships among three exposure indices were examined in the present study, using ICP-MS, where the sample was simply diluted. Thus the ICP-MS method was indicated to be useful for the determination of three exposure indices and therefore for the examination of the body lead burden.

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東京労災病院産業中毒センター
坂井 公

Reprint request:
Tadashi Sakai
Center of Occupational Medicine, Tokyo Labor Accident Hospital, 13-21, Omoriminami-4, Ota-ku, Tokyo, 143-0013, Japan

ICP-MSによる血漿，血液，および尿中鉛の測定と暴露指標としてのこれら3指標の関連

坂井　公，森田　陽子，荒木　高明
東京労災病院産業中毒センター

—キーワード—
鉛，血漿，誘導結合プラズマ質量分析計（ICP-MS）

要旨：誘導結合プラズマ質量分析計（ICP-MS）を用いた血漿，血液および尿中鉛の簡便・高感度な測定法を開発し，鉛の暴露指標としてのこれら3指標の関連について検討した．測定検体は男子鉛作業者（69名）の検診時に採取されたヘパリン血およびスポット尿である．ICP-MSによる測定では硝酸溶液で検体を希釈するが，血漿，血液，尿の希釈倍率はそれぞれ5倍，50倍，80倍である．血漿鉛，血液鉛，尿中鉛については従来の原子吸光光度方（AAS）による測定も行い比較した．血液鉛と尿鉛ではICP-MSによる値はAASによる値に近似していたが，AASによる血漿鉛濃度はICP-MSによる値の2倍以上となった．血液鉛の上昇につれて血漿鉛，尿中鉛とも指数的に増加した．血漿鉛と尿中鉛濃度との間には直線的な関係がみられた．

原　著

GC-MSによるトルエンジイソシアネート尿中代謝物の測定

森田　陽子[1]，坂井　　公[1]，Yangho Kim[2]

[1] 東京労災病院産業中毒センター，[2] Ulsan University Hospital

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要旨：トルエンジイソシアネート（TDI）作業者の生物学的モニタリングのために，TDIの尿中代謝物であるトルエンジアミン（TDA）の簡便な測定法を開発した．方法は，尿1mlに硫酸0.1mlを加えて1.5時間加水分解し，アルカリ化したのちジエチルエーテル中に抽出する．これに窒素を吹き付け蒸発濃縮したものをトルエンで溶解し，ヘキサフルオロ-n-酪酸無水物で誘導体化する．これを再び蒸発濃縮しトルエンに溶解し，ガスクロマトグラフ質量分析計で測定するものである．本法によりTDI暴露作業者32名の尿中TDAの測定を行ったところ，2,6-TDA濃度は0.1～28.5μg/l，2,4-TDAは0～5.1μg/lであった．一方，TDI非暴露者の尿中にはTDAはほとんど検出されなかった．

（日職災医誌，51：154—157，2003）

—キーワード—
トルエンジイソシアネート，トルエンジアミン，尿

はじめに

トルエンジイソシアネート（toluene diisocyanate）略してTDIは，刺激臭があり，常温では白色～淡黄色の液体である．TDIにはイソシアネート基の位置により6種類の異性体があるが，産業現場では2,4-，2,6-の2種類の異性体の混合物が使用されている．主な用途は，ポリウレタン樹脂，塗料，接着剤，ゴム，繊維処理剤等である[1,2]．TDIには皮膚や粘膜の刺激作用があり，皮膚に直接接触すると，化学火傷，接触皮膚炎を起こすほか，眼に暴露すれば流涙，炎症，角膜炎を生じることがある[3]．吸入した場合は咳，分泌亢進，胸痛，呼吸困難が現れるほか，重篤な場合では肺水腫を引き起こすことがある[4]．TDIには感作性があり，職業性喘息を引き起こす物質として知られている[5,6]．我が国でもTDIによる急性および慢性中毒例がいくつか報告されている．TDIは体内で代謝され，それぞれが対応するトルエンジアミン（toluenediamine，TDA）の異性体となるので[7]，これらを測定することによるTDIの生物学的モニタリングが可能と考えられる[8,9]．本研究では，超低濃度のTDAの測定法の開発をめざしてガスクロマトグラフ質量分析装置（GC-MS）を用いたTDAの簡易な分離測定法を検討し，実際の作業者に適用した．

GC-MS determination of urinary metabolite of toluene diisocyanate

材料と方法

対象はTDIを使用する楽器製造工場の作業者32名（暴露群）と非暴露者9名（対照群）のスポット尿である．採尿後すみやかに冷凍し，解凍は測定直前に行った．TDAの標準品は東京化成工業の2,4-TDA，3，4-TDA，2，6-TDAを用いた．内部標準物質のm-およびp-キシレンジアミン（m-，p- xylenediamine，m-，p-XDA）と誘導体化試薬のヘプタフルオロ-n-酪酸無水物（heptafluoro-n-butyric anhydride，HFBA）も同様である．ジエチルエーテル，水酸化ナトリウム，硫酸は和光純薬工業の特級試薬を用いた．試薬の調整や前処理にはMill-Qシステム（Millipore）による純水を使用した．

ふたつき遠心管（16ml）に尿1mlをとり，内部標準物質としてm-XDA，p-XDA混液（各2ppm）0.1mlと濃硫酸0.2mlを加えてよく混和し，90分間煮沸した．冷却後，8Nの水酸化ナトリウム溶液1mlを加えよく混和し，ジエチルエーテル2mlを加えて20分間振とう抽出した．5分間の遠心分離（3,000rpm）後に上層を分取し，窒素を吹き付け濃縮乾燥した．これをトルエン500μlで溶解し，HFBAを25μl加え55℃温浴中で1時間加熱しTDA，XDAを誘導体化した．これを再度窒素で濃縮乾燥してトルエン200μlに再溶解し，GC-MSによる測定に供した．

ガスクロマトグラフ（GC）には島津GC-17Aを用いた．カラムはJ&W社のDB-5（30m，内径0.32mm）を

使用し，サンプル1μlをスプリットレスで注入した．カラムは初温150℃で1分保持の後，毎分10℃で昇温し，終温280℃で1.5分保持した．質量分析計（MS）には島津QP5050Aを使用，インターフェース温度は280℃である．測定は陰イオン化学イオン化法（nagative ion chemical ionization：NCI）により選択イオン検出（selective ion monitoring：SIM）を行った．反応ガスにイソブタンを使用した．XDAの誘導体はm/z＝508，TDAの誘導体としてはm/z＝494を選択測定した．

結　果

まずジエチルエーテルによるTDAの抽出効率に及ぼす抽出液のpHの影響をみるために，抽出の前に加えるアルカリの量を検討した．図1に加水分解後の尿（約2ml）に種々の量の水酸化ナトリウム溶液（10N）と水を計2mlとなるように加えて抽出した時の，GC-MSクロマト上のTDAピーク面積の変化を示す．尿に添加したTDAの抽出では，水酸化ナトリウム（10N）が1.6ml，水0.4mlの場合が最も高いピーク面積となった．よってルーチン測定には加水分解後の尿（約1ml）に8Nの水酸化ナトリウム溶液1mlを加えることとした．TDAを水に添加した場合も同様の結果が得られた．一方，複数の尿にTDAを添加して測定を行ったところ回収率に違いがみられた．これらの結果より，尿検体の測定には標準添加法を用いることとした．また抽出溶剤にトルエンを使用したところ，抽出効率が低下した．

図2Aは尿に添加した2,6-TDAと2,4-TDAの検量線を示す．縦軸はTDAのXDA（内部標準物質）に対する比であり，TDAの濃度に対応して良好な直線性を示した．図2Bは水を用いた場合の検量線であり，尿と同様に良好な直線性が得られた．

図3に3種のTDAと2種のXDAのHFBA誘導体のイオンクロマトグラムを示す．Aは標準物質，Bは作業者の尿のクロマトグラムである．TDA 3種の誘導体の検出にはすべてm/z＝494を測定するが，3,4-，2,6-，2,4-の順に溶出するため，分離測定が可能である．XDA2種の誘導体はm/z＝508を用い，どちらも内部標準として用いることが可能である．TDA誘導体の保持時間は，2,6-TDAが7.4分，2,4-TDAは7.6分である．図Aの各標準物質の濃度は，TDAが100ppb，XDAは200ppbである．図Bの作業者尿には3,4-TDAは認められず，2,6-TDAの濃度は5.2μg/l，2,4-TDAは0.5μg/lである．

図4はTDA作業者（暴露群）32名と非暴露者（対照群）9名について尿中TDA濃度の測定結果を示す．検出限界は0.1μg/lである．暴露群の2,6-TDAの範囲は0.1～28.5（平均1.95）μg/l，2,4-TDAは0～5.1（平均0.79）μg/l，対照群のうち8名の2,6-TDA，2,4-TDA濃度は検出限界以下であり，1名のみが約0.1μg/lであった．

考　察

TDIには感作性があるためその許容濃度は非常に低く設定されている．日本産業衛生学会では，2,4-TDIと

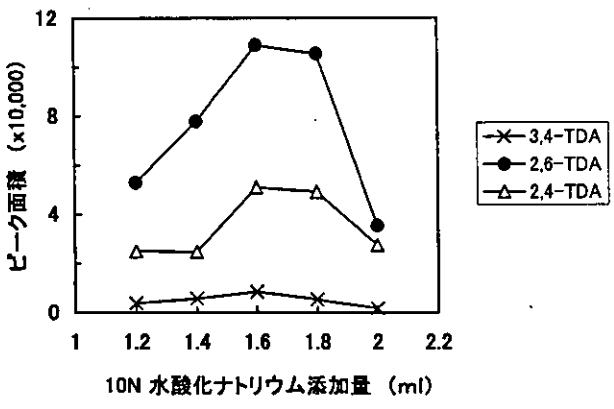

図1　TDA抽出時におけるアルカリ化の影響

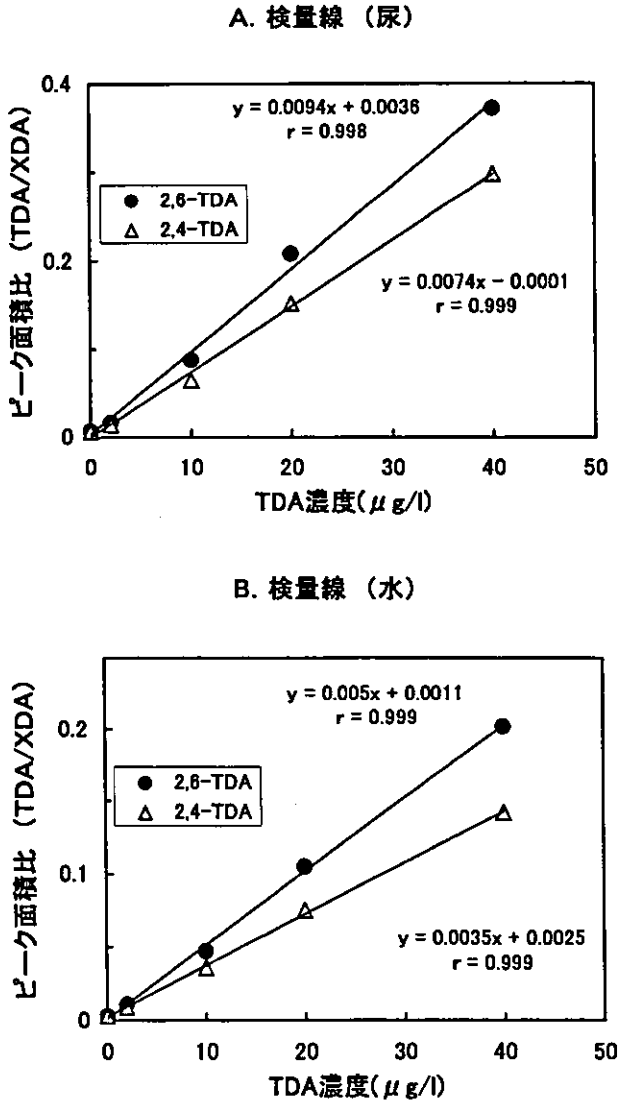

図2　直線性の検討