Bull. Environ. Contam. Toxicol. (1991) 47:797–803

Bulletin of Environmental Contamination and Toxicology

Simple Method for Determination of Methanol in Blood and Its Application in Occupational Health

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Methanol is one of the most popular solvents and its use in industrial and home-use solvent preparations has increased in recent years (Inoue et al. 1983; Kumai et al. 1983; Saito and Ikeda 1988). For the biological monitoring of exposure of humans as well as laboratory animals, blood is one of the biological samples for solvent analysis (e.g., see Lauwerys 1983 for a review on benzene and toluene). In practice, however, blood was seldom employed for methanol monitoring, primarily for technical complexities such as solvent extraction or head-space equilibration prior to gas chromatographic (GC) analysis.

In the present communication, a simple, and time- and labor-saving method of GC analysis for methanol is described. The confirmation of its validity in application to occupational health is also presented by means of comparison with breathing zone air analysis results after diffusive sampling (Berlin et al. 1987; Kawai et al. 1990), and with results of urinalysis for methanol (Kawai et al. 1987). The method is probably applicable also to the analysis for various organic solvents in blood and urine.

MATERIALS AND METHODS

Blood and urine samples were obtained from 19 factory workers of both sexes (8 men and 11 women), who were engaged in the production of pocket methanol fuel (jelly-like methanol-impregnated material to be used for e.g. out-door cooking), and were exposed to up to several thousand ppm methanol. They were invited to an examination room at the end of the workshift, and blood samples were taken within 5 to 10 min after the departure from their workshops. Then, they were asked to visit restrooms for urine sampling.

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Methanol in blood was measured as follows: 2 ml of blood was drawn from cubital vein with a 2-ml clinical disposable plastic syringe (Terumo and Co., Osaka, Japan) and anticoagulated by gentle mixing with dipotassium EDTA in Sysmex® (2 ml bottle: Toa Medical Electronics Co., Kobe, Japan). The samples were kept refrigerated and analyzed within 24 hrs. An aliquot, 2 µl per injection, was introduced without any further pretreatment to a GC system for methanol analysis. The GC used was Shimadzu GC-7 AG equipped with a 3mm x 4 m glass column (packed with 10% SBS 100 on Shimalite TPA, 60-80 mesh), and connected with a FID-detector and a data processor. The detector and the oven were heated at 180°C and 60°C, respectively. Between the detector was connected a glass insert (Shimadzu glass insert type G) in which ca. 4 mg quartz wool for GC column was packed in a length of ca. 5 mm. The glass insert is stained when blood is injected into GC and should be replaced with a new one after some 50 blood injections. Care should be taken to inject quickly to avoid heat-coagulation of blood in the injection needle.

The analysis of urine for methanol (Kawai et al. 1987) was as previously described. A newly developed plastic sampler with water as absorbent (Uchida et al. 1989) was employed for diffusive sampling of breathing zone air for the measurement of time-weighted average intensity of methanol exposure during an 8-hr shift (Kawai et al. 1990).

RESULTS AND DISCUSSION

Typical chromatograms are presented in Fig. 1 to show that there was only a small peak (possibly of acetone) in the blood from a nonexposed which might interfere methanol determination, and that the retention time for methanol peak in the blood of an exposed worker is identical with that for authentic methanol dissolved in water. When both water and blood from nonexposed subjects were spiked with known amount of methanol and analyzed as described in MATERIALS AND METHODS, the peak space was proportional to the amount of methanol added (Fig. 2) and the two calibration curves (one with water and the other with blood) were identical to each other. GC analysis of the same blood (spiked with 39.5 µg methanol/ml blood) but with various amount (i.e., 1 to 5 µl blood) also showed that the methanol peak space was linearly related to the amount of blood injected (Fig. 3). When each of 5 blood samples from exposed workers were measured 5 times, the coefficient of variation was <4%. Thus, it was concluded that methanol in blood is measurable by the method developed. It should be added that each

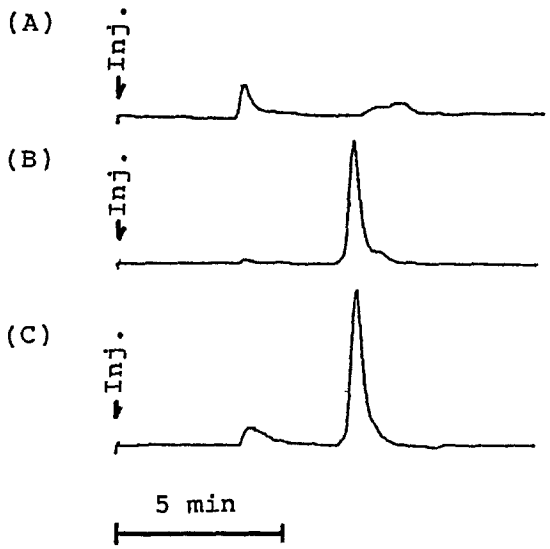

Figure 1. Typical chromatograms of (A) blood from a nonexposed subject (no methanol added), (B) water spiked with authentic methanol (30.0 µg/ml), and (C) blood from a subject (exposed to 18.5 ppm methanol as an 8-hr time-weighted average; 43.4 µg/ml methanol was detected in blood). Inj.: Injection.

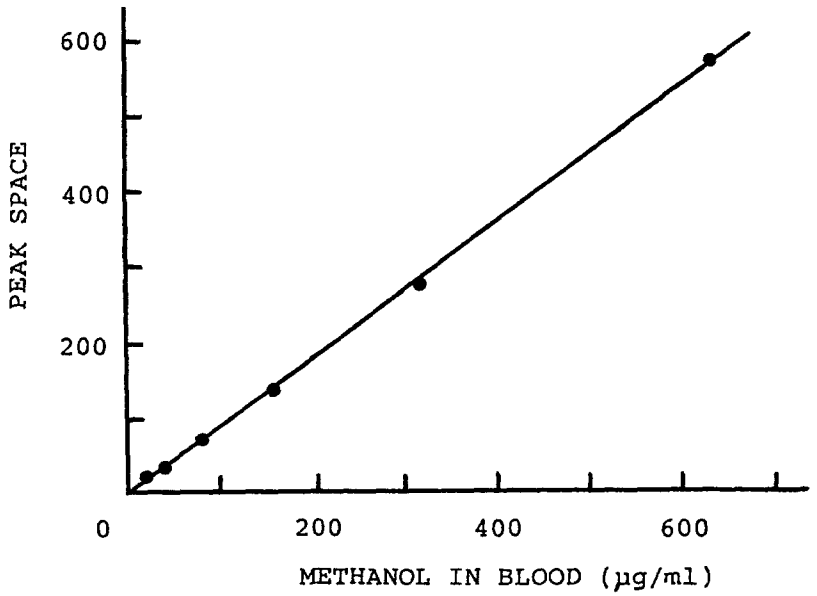

Figure 2. The linear correlation to show that the space of the methanol peak (in an arbitrary unit) was proportional to the amount of methanol added. Two µl blood was injected per determination.

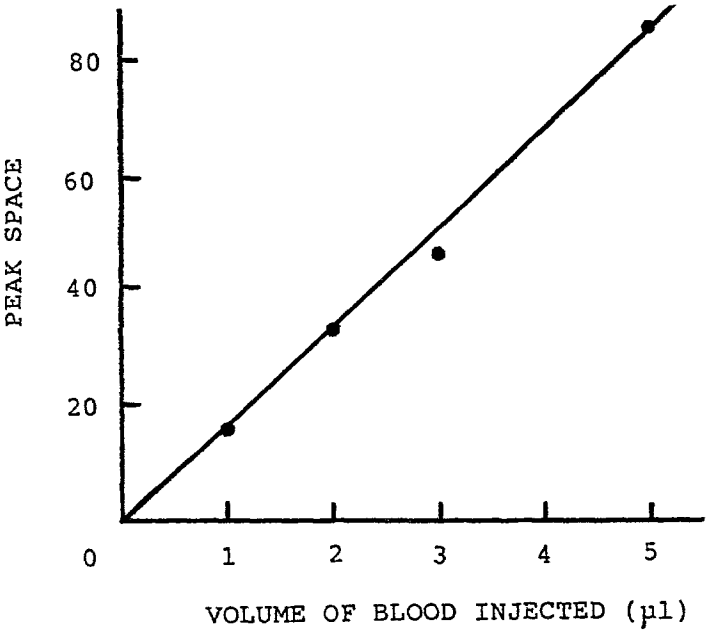

Figure 3. The linear relationship between the amount of blood injected (1 to 5 µl/injection) and the space of the methanol peak (in an arbitrary unit) detected.

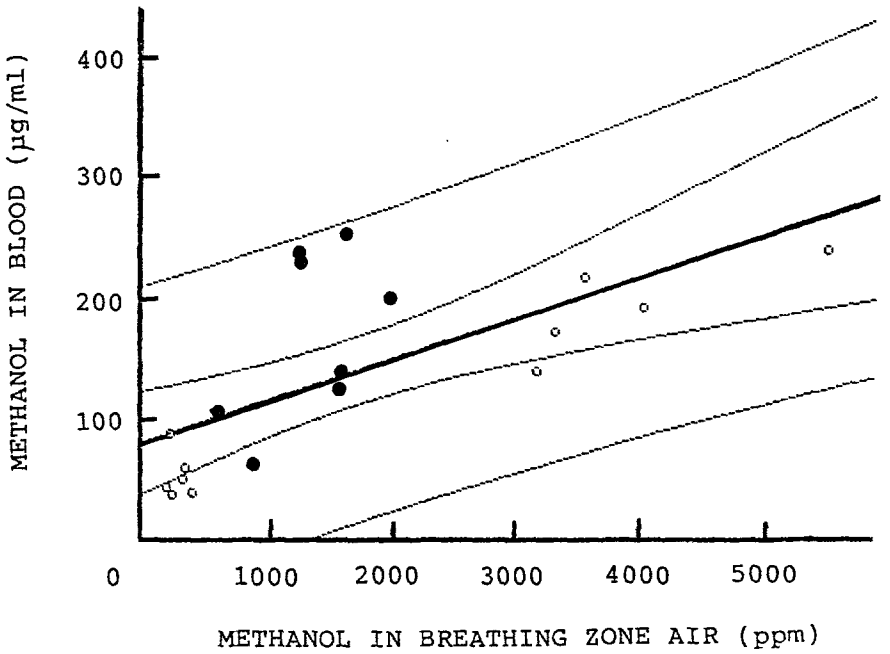

Figure 4. The relationship between the methanol in blood and time-weighted average methanol concentration in breathing zone air. The open and solid circles indicate individual men and women, respectively.

Table 1. Correlation among ethanol in breathing zone air, methanol in blood and methanol in urine

Sex	Matrices compared[a]	Parameters[b] α	ß	r
Men and women combined (19 subjects)				
	Air - Blood	79.5	0.034	0.683**
	Air - Urine	149.5	0.051	0.715**
	Blood - Urine	132.9	0.748	0.529*
Men only (8 subjects)				
	Air - Blood	57.9	0.081	0.516*
	Air - Urine	90.7	0.062	0.399*
	Blood - Urine	14.6	0.957	0.963**
Women only (11 subjects)				
	Air - Blood	42.4	0.038	0.963**
	Air - Urine	191.6	0.045	0.787**
	Blood - Urine	140.1	1.208	0.820**

a The media compared are breathing zone air, and blood and urine taken at the end of the 8-hr shift. For regression analysis, the former is taken on X axis, and the latter on Y axis. For example, air - blood means that methanol in air is on X axis and methanol in blood on Y axis.

b Parameters of the calculated regression lines so that $Y=\alpha+\beta X$. The unit is ppm for methanol in air, and μg/ml both for methanol in blood and in urine. r shows the correlation coefficient. The asterisks indicate statistical significance of the correlation coefficient (** for $p<0.01$ and * for $p<0.05$).

analysis needs less than 10 min, and about 50 samples can be readily analyzed in a day including the time for the exchange of a glass insert.

Methanol concentration in blood correlates significantly ($p<0.01$) with the time-weighted average concentration of methanol in breathing zone air as monitored by diffusive sampling during an entire shift (Fig. 4 and Table 1) as expected. Further perusal of the figure suggests that the slope of the regression line might be different between the two sexes. Separate calculation for men and women in fact shows that the slope is twice as large for men as for women (Table 1). This may be a reflection of larger breathing capacity and less fat tissue in men than in women. The correlation of methanol concentration between in air and in shift-end urine is higher in women than in men. Methanol concentration in the blood of 10 men with no known exposure to methanol was 1.51±0.33 μg/ml (mean±standard deviation; with an

assumption of normal distribution) or 1.47 μg/ml (1.267) (geometric mean and geometric standard deviation in parenthesis; with an assumption of log-normal distribution).

Of particular interest is the relation of methanol in blood with that in urine. As early as in 1952, Leaf and Zatman made a volunteer experiment in which three subjects were given 71 or 84 mg methanol/kg orally, and methanol in blood and urine were colorimetrically measured after oxidation to formaldehyde. The study showed that the ratio of methanol in blood/that in urine was rather constant at 1.3 at the two doses and at 2 to 5 hr after the ingestion. In the present study, the slope of the regression line taking methanol in blood and in urine on X and Y axis, respectively, is 0.748 (Table 1). In other words, the blood/urine ratio is 1.34 in close agreement with the observation by Leaf and Zatman (1952), despite the difference in the route of administration and in the analytical method employed. In practice, it is necessary for biological monitoring of methanol by means of urinalysis, and even more so when blood was analyzed, to take rapid biological half-time of methanol into consideration. The data presented by Leaf and Zatman (1952) suggest that the half-time for methanol in blood and urine is less than 2 hrs, whereas Kawai et al. (1988) observed that the half-time for urinary methanol is about 8 hr in close agreement with the observation by Tada et al. (1974). Thus, the timing of blood sampling, i.e., immediately after the termination of exposure, is of critical importance for precise reflection of exposure intensity.

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Received July 3, 1990; accepted July 4, 1991.