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Formic acid excretion in comparison with methanol excretion in urine of workers occupationally exposed to methanol

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Summary. A semiautomated head-space gas chromatographic (GC) method was developed for measuring formic acid in urine. The method consists of heating 1 ml urine sample in a 20-ml air-tight vial in the presence of 1 ml sulfuric acid and 2 ml ethanol at 60°C for 30 min for ethyl esterification and air-liquid equilibrium, followed by automatic injection of 1 ml head-space air into a flame ionization detector GC. The detection limit was 1 mg/l for formic acid. The method was applied to measure formic acid in the shift-end urine samples from 88 workers exposed to methanol at 66.6 ppm (as geometric mean) and in urine samples from 149 nonexposed controls. Methanol concentrations were also determined. Regression analysis showed that urinary formic acid concentrations, as observed or corrected for either creatinine concentration or specific gravity of urine (1.016), correlated significantly with time-weighted average intensities of exposure to methanol vapor. Men excreted significantly more formic acid than women. Comparison with methanol excretion suggested, however, that urinary formic acid is less sensitive than urinary methanol as an indicator of methanol vapor exposure, primarily because the background level for formic acid (26 mg/l as arithmetic mean, or 23 mg/l as geometric mean) is more than ten times higher than the level for methanol (1.9 mg/l as arithmetic mean, or 1.7 mg/l as geometric mean). After theoretical methanol exposure at infinite concentration, the urinary formic acid/methanol ratio should be about 0.4.

Key words: Biological monitoring – Formic acid excretion – Methanol exposure – Methanol excretion – Urinalysis

Introduction

In accordance with the popular use of methanol for industrial purposes (Inoue et al. 1983; Kumai et al. 1983) and in consumers products (Saito and Ikeda 1988), increasing efforts have been made to develop an effective method for biological monitoring of exposure to this solvent, which is unique in high hydrophilicity. In practice, two exposure-related chemicals in urine, methanol per se (Tada et al. 1974, 1975; Dutkiewicz et al. 1980; Sedivec et al. 1981; Kawai et al. 1991a) and formic acid (Mráz et al. 1978; Ferry et al. 1980; Triebig and Schaller 1980; Liesivuori and Savolainen 1987), have been investigated in addition to methanol (Heinrich and Angerer 1982; Kawai et al. 1991b) and formic acid (Baumann and Angerer 1979; Heinrich and Angerer 1982; Lee et al. 1992) in blood. Trials were also made to evaluate urinary formic acid as an indicator of exposure to environmental formaldehyde (Heinzow and Ellrott 1992).

Considering noninvasive urine collection as more advantageous than inevitably invasive blood sampling, trials were made in the present study to establish a hand-saving semiautomatized head-space gas chromatographic (GC) method for urinary formic acid. The method developed was used in occupational health to determine formic acid level in the urine of methanol-exposed workers and nonexposed controls. The results presented here evaluate urinary formic acid determination in comparison with the measurement of methanol in urine as a tool for the biological monitoring of occupational exposure to methanol.

Materials and methods

Study population

A survey of two factories, factory A and factory B, were conducted in the second half of working weeks. The workers in factory A engaged in the production of pocketable methanol fuel to be used, for example, for picnic. Those in factory B were plastics-producing workers. Details of occupational health data on methanol exposure and health of the workers have been described elsewhere (Kawai et al. 1991a, 1992a). Their jobs in association with methanol exposure are summarized in Table 1. In total, 88 exposed subjects (53 men and 35 women) were studied for 8-h time-weighted average (TWA) methanol vapor exposure and for formic acid and methanol concentrations in shift-end urine. For compari-

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Table 1. Study population by sex and age

Group	Workplace	Sex	*n*	Methanol exposure		Exposure to other solvents (GM)
				Jobs	GM (GSD)	
Exposed	Factory A	Men	20	Production of semisolid fuel[a]	262 (5.47)	None
		Women	28		223 (5.06)	
		Total	48		238 (5.24)	
	Factory B	Men	33	Manual washing of brushes[a]	16 (3.43)	Toluene (7.3)
		Women	7		9 (1.91)	Styrene (4.7)
		Total	40		14 (3.22)	
	Sum of factories A and B	Men	53		46 (7.18)	
		Women	35		118 (7.02)	
		Total	88		67 (7.52)	
Nonexposed controls[b]		Men	79			
		Women	70			
		Total	149			

GM, Geometric mean (ppm); GSD, geometric standard deviation
[a] With protective gloves
[b] Office workers

son, 149 control subjects (79 men and 70 women) offered urine samples for formic acid and methanol analyses.

Measurement of methanol vapor concentration

The measurement of TWA intensity of exposure to methanol vapor was conducted with diffusive samplers (with water as absorbent; Uchida et al. 1990) as previously described (Kawai et al. 1991a).

Analyses of urine for formic acid and methanol

The exposed workers were asked to pass urine at 1400–1500 hours of the study day, and urine samples were collected near the end (i.e., at 1600–1700 hours) of an 8-h working shift. Measurement of methanol was conducted as previously described (Kawai et al. 1991a).

Urinalysis for formic acid was carried out by means of head-space GC after conversion to ethyl formate. Analytical conditions are detailed below (see "Results") and can be summarized as follows. Under standard conditions established in the present study, 1 ml urine sample was taken in a 20-ml vial (for head-space GC analysis; Hewlett Packard, Philadelphia PA, USA) together with 2 ml ethanol and 1 ml concentrated sulfuric acid, and the vial was immediately sealed with a Teflon-coated silicone septum. The vial was heated at 60°C for 30 min in an oil bath for ethyl esterification and air-liquid equilibrium of ethyl formate vapor thus formed, and 1 ml head-space air was injected by means of an automatic air sampler (Hewlett Packard Model 19395A) into a flame ionization detector (FID) GC (Hewlett Packard Model 5890 II).

The GC was equipped with a DB-WAX capillary column (J & W, Folson CA, USA; diameter 0.53 mm × 60 m, with a film thickness of 1.0 μm). Helium, a carrier gas, was allowed to flow at 59 g/cm^2. Supply of air, H_2 and N_2 to the FID was at 2.8, 1.4, and 5.0 kg/cm^2, respectively. The oven, injection port, detector, and loop connecting the antosampler and GC were kept at 60°, 150°, 200°, and 100°C, respectively. A splitless mode was employed. The detection limit for ethyl formate under the study conditions was about 1.6 mg (1.0 mg as formic acid)/l urine. Analysis took 30 min per sample.

The urinary concentrations of formic acid and methanol were expressed as observed (i.e., without correction) or after correction for creatinine concentration (Jackson 1966) or for a specific gravity of urine of 1.016 (Rainsford and Lloyd Davies 1965). Creatinine concentration and specific gravity were measured by conventional methods of colorimetry and refractometry, respectively.

Gas chromatographic – mass spectrometric identification of ethyl formate

A GC (Hewlett Packard 5890 A) in connection with a mass-selective detector (Hewlett Packard 5970 B) was used. A DB-WAX column for GC was 30 m in length, 0.25 mm in inner diameter and 0.5 μm in film thickness. The oven, the GC injector, and a mass-selective detector source were heated at 35°, 250°, and 250°C, respectively. A split ratio of 10:1 was employed for GC. Ion mass spectra were obtained in a scanning mode (5.71 scans/s in a range of 25–100) at 70 eV. The data were processed in a work station (Hewlett Packard 59970 A) using the NBS Mass Library. For head-space GC analysis, the vial was heated at 60°C for 30 min for equilibrium, and 1 ml sample air was introduced to the GC - mass spectrophotometry system.

Reagents

Ethanol, concentrated sulfuric acid (98%), formic acid and ethyl formate were of the best grade available (Wako Pure Chemicals, Osaka, Japan).

Statistical analysis

Regression analysis was employed. The significance of difference in the slopes of regression lines was examined by the *F* test.

Results

Establishment of the method for determination of formic acid in urine

The GC analysis of formic acid requires derivatization of the acid to a more volatile compound. In the present study this was achieved by conversion of the acid to ethyl formate by heating in the presence of ethanol and sulfuric acid after Smallwood (1978). The optimum condi-

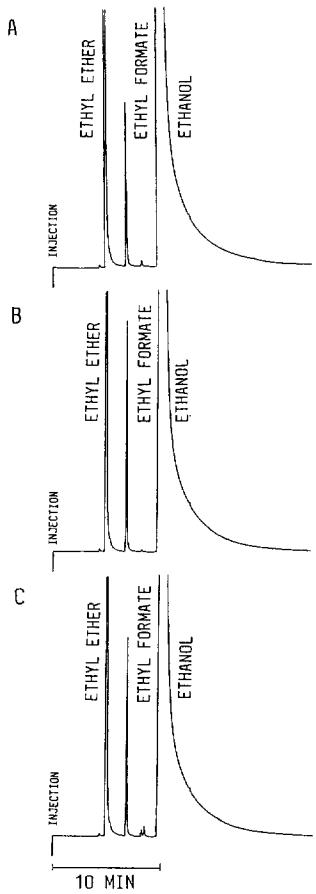

Fig. 1. Gaschromatograms for ethyl formate determination. (**A**) The chromatogram of authentic ethyl formate, (**B**) the chromatogram of authentic formic acid after reaction with ethanol, and (**C**) the chromatogram obtained by the treatment of a urine sample from a worker exposed to methanol at 1626 ppm, after reaction with ethanol

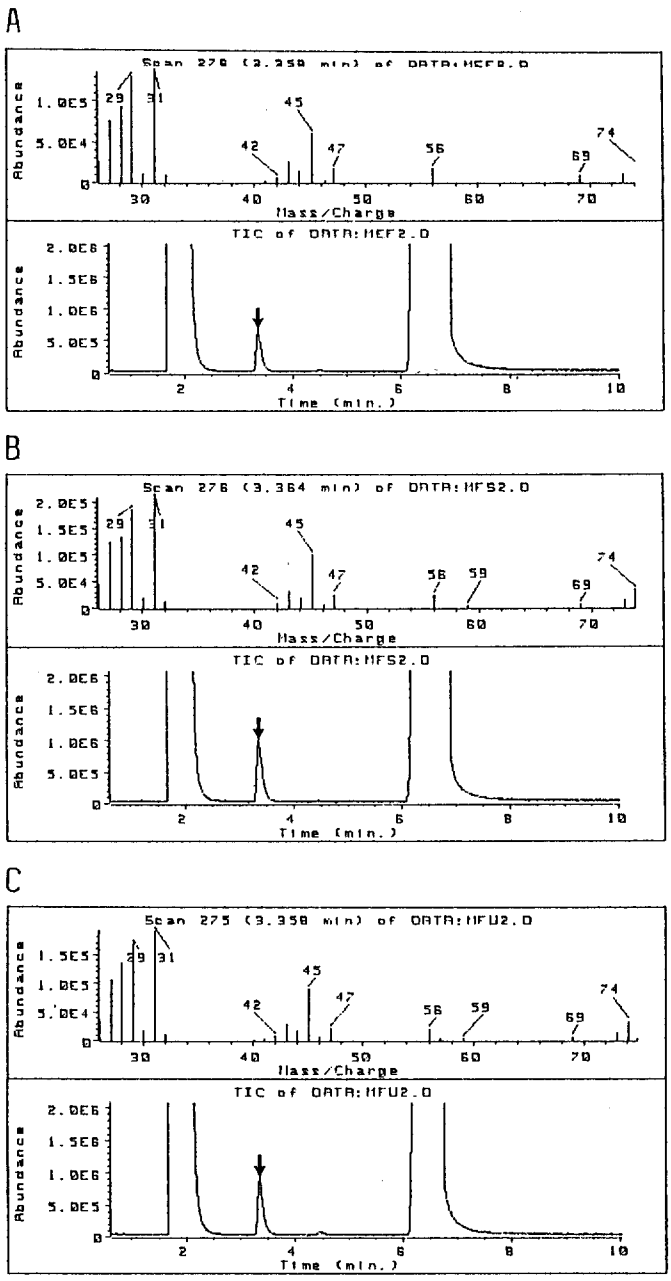

Fig. 2. Gaschromatographic-mass spectrometric identification of ethyl formate. (**A**) The mass spectrum of authentic ethyl formate, (**B**) the mass spectrum of authentic formic acid after reaction with ethanol, and (**C**) the mass spectrum of a urine sample from a worker exposed to methanol at 1626 ppm, after reaction with ethanol

tions for esterification were investigated by the stepwise change of the amounts of sulfuric acid and ethanol in the vial. First, authentic formic acid (2.595 µmol or 119.45 µg; equivalent to 192.24 µg ethyl formate) in 2 ml ethanol was mixed with 0.50–2.25 ml sulfuric acid, and the volume was adjusted with water to a final volume of 5 ml. Analysis under the standard head-space GC conditions showed that the reaction needed 1.0–1.5 ml sulfuric acid for maximal esterification. In contrast, changes in the amount of ethanol up to 3.0 ml in presence of 1 ml sulfuric acid did not result in any significant variation in the amount of ethyl formate formed as long as 1.0 ml or more ethanol was present.

It was further observed that the presence of sulfuric acid is important for the air-liquid equilibrium. When authentic ethyl formate was heated with ethanol at various amounts (i.e., 0.25–3.0 ml, with a total volume being adjusted to 5 ml with water) in absence of sulfuric acid, the amount of ethyl formate detected in the head-space air decreased as more ethanol was added. In the presence of 1 ml sulfuric acid, however, the concentration of ethyl formate in the head-space air did not change regardless of variation in ethanol amount in a range of 1–3 ml.

Typical chromatograms are depicted in Fig. 1 to show that the authentic ethyl formate was clearly separated from ethanol (Fig. 1A), that the retention time of the product from formic acid and ethanol was identical to that of authentic ethyl formate (Fig. 1B), and that essentially the same chromatogram was obtained by the treatment of a urine sample from a methanol-exposed worker with no confounding peaks in the chromatogram (Fig. 1C). The column selected (a 60-m-long DB-WAX column) allowed the appearance of a peak for ethyl formate prior to a large peak of ethanol so that the determination of the former was not disturbed by the latter. A long tailing of the latter peak was terminated in 30 min when the column temperature was kept at 60°C. The ethyl formate

Table 2. Concentrations of formic acid and methanol in urine of nonexposed subjects

Compound (Sex)	Number	Observed values		Values corrected for			
				Creatinine		Specific gravity (1.016)	
		AM ± ASD	GM (GSD)	AM ± ASD	GM (GSD)	AM ± ASD	GM (GSD)
Formic acid							
Men	79	26.36 ± 12.98	23.37 (1.65)	17.41 ± 12.08	14.64 (1.77)	20.20 ± 10.02	18.02 (1.62)
Women	70	25.96 ± 11.18	23.46 (1.61)	28.84 ± 11.32	26.52 (1.53)	19.86 ± 6.32	18.79 (1.42)
Total	149	26.17 ± 12.17	23.41 (1.63)	22.78 ± 13.04	19.36 (1.80)	20.04 ± 8.49	18.38 (1.53)
Methanol							
Men	79	2.10 ± 0.97	1.85 (1.73)	1.44 ± 0.93	1.16 (2.02)	1.68 ± 0.91	1.42 (1.83)
Women	70	1.65 ± 0.81	1.46 (1.66)	2.09 ± 1.55	1.65 (1.97)	1.40 ± 0.88	1.17 (1.82)
Total	149	1.89 ± 0.93	1.65 (1.72)	1.74 ± 1.30	1.37 (2.04)	1.54 ± 0.91	1.30 (1.84)

Values in the table are arithmetic mean (AM) ± arithmetic standard deviation (ASD), and geometric mean (GM) [geometric standard deviation (GSD) in parenthesis]. The unit of the values (except the number of subjects, and for GSD which is dimensionless) are mg/l for observed values and values corrected for a specific gravity of urine of 1.016, and mg/g creatinine for values corrected for creatinine

derived from a urine sample of a worker exposed to methanol at 1626 ppm was further identified in comparison with the authentic ethyl formate by GC-mass spectrophotometry as shown in Fig. 2. The mass spectra, one from authentic formic acid (Fig. 2B) and the other from a urine sample of a methanol-exposed worker (at 1626 ppm; Fig. 2C) both after reaction with ethanol, were identical with that of authentic ethyl formate (Fig. 2A).

The calculation line was thus established with 2 ml ethanol and 1 ml sulfuric acid together with 1 ml formic acid solution in water at the concentrations of 1.08–8.69 m*M* (50–400 mg/l). The experiment showed that there is a linear relation between the formic acid concentrations and the space of the peak for ethyl formate in the chromatograms. Addition of authentic ethyl formate to several urine samples made it clear that the slope of the regression line stays unchanged independently of replacement of water with urine. The repeated analysis of 200 mg/l formic acid in water showed that the coefficient of variation was 0.6% (five determinations). When five urine samples (endogenous formic acid concentration; less than 40 mg/l) from five nonexposed subjects were spiked with 200 mg/l formic acid [by the addition of 0.1 ml of 2000 mg/l formic acid (or water) to 0.9 ml each of the urine samples], and the urine samples (both spiked and nonspiked) were analyzed for formic acid, the average recovery of the formic acid added was 98.7% ± 3.0% [arithmetic mean (AM) ± arithmetic standard deviation (ASD) with a coefficient of variation of 3.1%; five determinations].

Distribution of formic acid and methanol in urine from nonexposed subjects

To investigate the background level of formic acid in urine, urine samples from 149 nonexposed people (79 men and 70 women) were subjected to the head-space GC analysis. Methanol concentrations were also determined in the same urine samples. The results are summarized in Table 2. It was found that the coefficients were sometimes more than 50% both in formic acid and methanol concentrations, when AM and ASD values were calculated assuming a normal distribution. Accordingly, geometric means (GM) and geometric standard deviations (GSD) were also calculated with a log-normal distribution assumption. GSD values were about 2 or less in all cases.

It is evident from Table 2 that ten or more times formic acid than methanol is excreted in the urine of the nonexposed subjects. There was no apparent sex difference either in formic acid levels or in methanol levels (Table 2).

Increase in concentrations of formic acid and methanol in urine as a function of higher methanol vapor exposure

The data of 132 men and 105 women on TWA methanol vapor exposure concentrations and concentrations of formic acid and methanol in shift-end urine samples were subjected to regression analysis. The urinary concentrations were expressed as observed and also after correction for urine density by means of creatinine concentration and specific gravity (1.016). The results are summarized in Table 3 in terms of the slopes and the intercepts on the vertical axis, together with correlation coefficients and statistical significance. It is evident that formic acid in shift-end urine correlates significantly with TWA intensity of methanol vapor exposure, independently of sex and urine density correction. The same is also the case for urinary methanol. It should be noted, however, that the intercept on the vertical axis was generally larger for formic acid than for methanol (Table 3). The scatter diagrams for formic acid and methanol are depicted in Figs. 3 and 4, respectively; men and women were treated separately because there was a significant difference ($P < 0.01$–0.05, depending on the cases) between the slopes of the regression line for men and that for women both in formic acid and methanol regardless of correction for urine density (Table 3).

Table 3. Parameters of regression line between TWA intensity of exposure to methanol and excretion of formic acid or methanol in shift-end urine

Sex	Correction for urine density	Formic acid			Methanol		
		α^a	β^a	r^b	α^a	β^a	r^b
Men ($n = 132$)	Observed value	0.089**	25.16	0.798	0.119**	2.75	0.924
	Value corrected for creatinine	0.110**	20.76	0.643	0.128*	3.11	0.764
	Value corrected for specific gravity (1.016)	0.095**	21.05	0.659	0.118**	2.87	0.784
Women ($n = 105$)	Observed value	0.049	28.84	0.888	0.092	14.70	0.837
	Value corrected for creatinine	0.048	29.32	0.841	0.091	13.42	0.735
	Value corrected for specific gravity (1.016)	0.029	21.17	0.832	0.052	9.97	0.813

Asterisks indicate that the difference between two sexes is statistically significant: **, $P < 0.01$; *, $P < 0.05$

[a] α and β are the slope and the intercept on the Y axis of the calculated regression line so that $Y = \alpha X + \beta$, where Y is formic acid or methanol concentration in urine (units: mg/l for observed values and values corrected for specific gravity of urine of 1.016, and mg/g creatinine for creatinine-corrected values), and X is TWA methanol concentration (units: ppm) in breathing zone air

[b] Correlation coefficient; all the coefficients are statistically significant ($P < 0.01$)

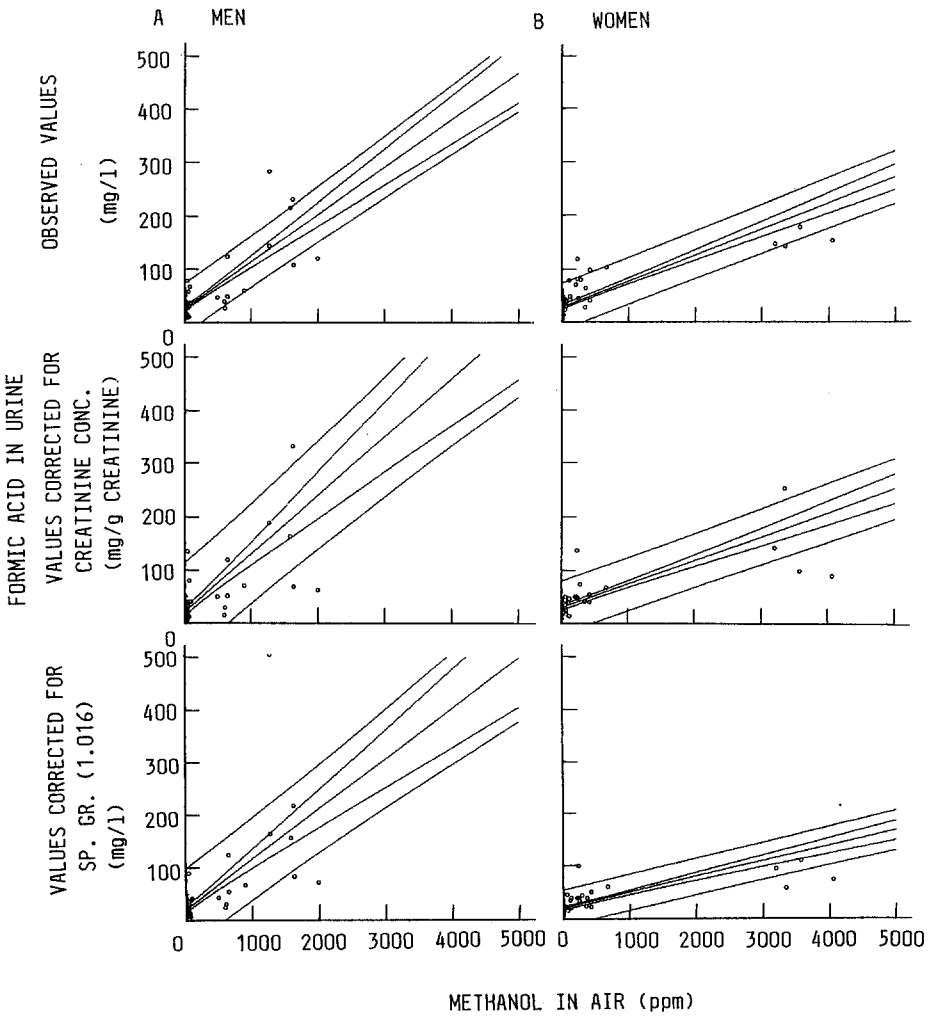

Fig. 3. Quantitative correlation between TWA intensity of exposure to methanol vapor and excretion of formic acid in shift-end urine. The line in the middle is a calculated regression line. The *inner* curves represent the 95% confidence range of the regression line, and the *outer* curves indicate that for individual values. Each circle represent an individual case. The parameters of the regression lines are given in Table 3, by sex and by correction for urine density

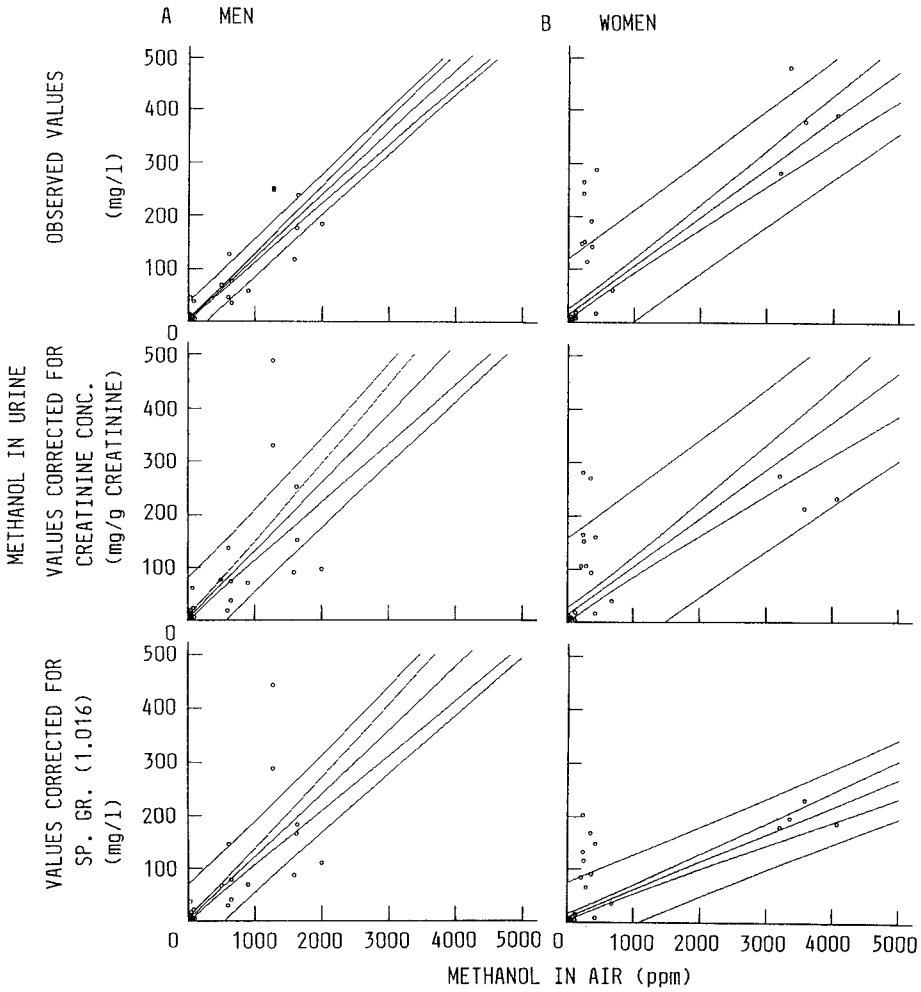

Fig. 4. Quantitative correlation between TWA intensity of exposure to methanol vapor and excretion of methanol in shift-end urine. Notes are as under Fig. 3

Discussion

The methods employed in previous studies to measure urinary formic acid were various. In the study by Angerer (1976), formic acid was decomposed to carbon monoxide in the presence of sulfuric acid, and the carbon monoxide thus formed was further reduced to methane on a catalytic column before the measurement by a flame inonization detector. Smallwood (1978) developed a method to measure formic acid after ethyl esterification in the presence of sulfuric acid and ethanol. Bricknell and Finegold (1978) first esterified formic acid to its methyl ester by an overnight reaction with a trifluoride methanol reagent, and then converted the ester to dimethylformamide by the reaction with dimethylamine, prior to determination by GC. Quite differently from these chemical pretreatments, Triebig and Schaller (1980) developed an enzymic method, in which formic acid was oxidized by formate dehydrogenase in the presence of NAD; the amount of formic acid present was measured by the appearance of NADH absorption at a wavelength of 340 nm. Ogata and Iwamoto (1990) took advantage of the same enzymic reaction in concert with NAD diaphorase and *p*-indonitrotetrazolium violet (INT), so that red color due to the reduction of INT was measured at 500 nm; the intensity of the color was proportional to the amount of formic acid. A similar colorimetric method was employed by Lee et al. (1992) for the detection of possible accumulation of formic acid in blood after experimental exposure of volunteers to methanol vapor.

In the present study the method of Smallwood (1978) was considered most suitable for the development of a semiautomated head-space GC method, and the results were quite satisfactory, as previously detailed. In the original method, Smallwood (1978) added 1.56 ml ethanol and 0.44 ml sulfuric acid per milliliter of a sample solution, and the mixture was heated at 55°C for 30 min. More sulfuric acid (1 ml) was added to 1 ml urine sample to obtain maximal esterification in a shorter time (30 min) but at a higher temperature (60°C), although 2 ml ethanol may be more than enough. The method thus developed allows man-free operation of the GC system for the determination of urinary formic acid; once ethanol and sulfuric acid are properly added, it is possible to determine formic acid in urine at a rate of 2 samples/h, or almost 50 samples daily. Although 30 min might appear rather long as the time necessary for one chromatographic analysis, this coincides with the time necessary for esterification followed by air-liquid equilibrium.

Formic acid is known to be present in the urine from nonexposed subjects. For example, Triebig and Schaller (1980) observed that formic acid concentration ranged 2

to 30 mg/l in the urine of 20 Europeans (both sexes combined), and a similar study by Heinrich and Angerer (1982) showed that formic acid concentration was in a range of 3–50 mg/l in the urine from 26 male Europeans. In more recent stdies, Ogata and Iwamoto (1990) found that formic acid concentration in the urine from 31 nonexposed male Japanese was 7.82 mg/g creatinine as GM (GSD 1.94), whereas 60 mg/l was reported as the 95% percentile value for urinary formic acid among Europeans; the distribution observed was apparently not normal (Heinzow and Ellrott 1992). The present observations on 149 Japanese men and women (Table 2) are in close agreement with the findings of Triebig and Schaller (1980), Heinrich and Angerer (1982), and Heinzow and Ellrott (1992) and are somewhat higher than those of Ogata and Iwamoto (1990).

An important point of evaluation is the comparison of methanol and formic acid both in urine as an indicator of occupational methanol exposure. The authors of early studies (e.g., Mráz et al. 1978; Baumann and Angerer 1979) were skeptical on the value of urinary formic acid as an indicator of occupational exposure to methanol. Mráz et al. (1978) in a study of an experimental vapor exposure of volunteers to methanol at 76 and 155 ppm for 8 h concluded that urinary formic acid level cannot be utilized for the purpose of exposure tests. Baumann and Angerer (1979), after investigating urinary formic acid levels in 20 workers exposed to up to 134 ppm methanol, concluded that they could not find a clear correlation between methanol exposure and resulting formic acid levels in urine. Ferry et al. (1980) in their oral administration experiment of methanol to volunteers also concluded that urinary formic acid level was too variable to be of value. In a recent study in which human volunteers were exposed to 200 ppm methanol for 6 h, Lee et al. (1992) found that formic acid in blood stayed unchanged after exposure, although urinary methanol levels were markedly elevated.

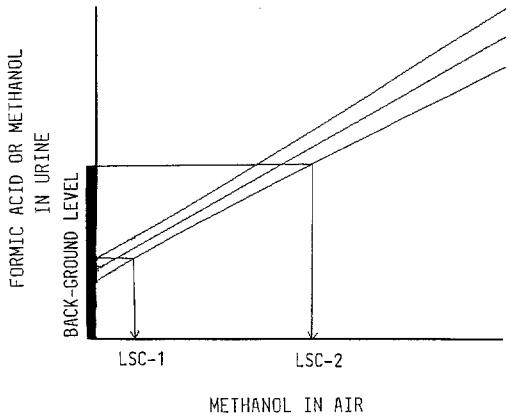

Fig. 5. Graphic presentation of the strategy to measure sensitivity of urinalysis as a tool of biological monitoring of low dose methanol vapor exposure. The line in the middle is a calculated regression line. The curves on both sides of the line represent the 95% confidence range of the regression. The shaded bar alongside the vertical axis shows the level in the urine of the nonexposed subjects, or 'the back-ground level' (for values, see Table 2). *LSC,* lowest separable concentration; LSC-1 and LSC-2 are the methanol concentration at which the exposed can be separated from the nonexposed, by the comparison with the 95% upper limit at 0 ppm, or with the 95% upper limit of the 'back-ground level', respectively

Table 4. The lowest concentration at which the exposed can be separated from the nonexposed by means of urinalysis for formic acid and methanol

Analysis for	Separation by			
	LSC-1 (ppm)		LSC-2 (ppm)	
	Men	Women	Men	Women
Formic acid	110	190	524	768
Methanol	55	222	45	–[a]

For the definition of LSC-1 and LSC-2, see Fig. 5

[a] The value cannot be determined because the 95% lower limit of the intercept on the vertical axis is larger than the 95% upper limit of the background level

Liesivuori and Savolainen (1987), however, observed a significant correlation ($r = 0.81$, $P < 0.01$) between methanol vapor concentration (up to ca. 160 ppm as monitored by silica gel tubes) and formic acid concentration (corrected for creatinine concentration) in the shift-end urine samples when they investigated 13 methanol-exposed workers (assumedly men).

Rather few authors have investigated both urinary formic acid and methanol in parallel. Heinrich and Angerer (1982) analyzed formic acid and methanol levels in the urine (collected over an afternoon shift) in addition to methanol in blood among 20 male pesticide-synthesizing workers who were exposed to methanol at 37 to 231 ppm (93 ppm as GM). The AM ± ASD for urinary formic acid was 29.9 ± 28.6 mg/l, in contrast to 21.8 ± 20.0 mg/l for urinary methanol. By the comparison with the levels in the urine from the nonexposed, Heinrich and Angerer (1982) concluded that urinary methanol concentration has a better sensitivity as biological exposure indicator than does formic acid, although overlapping ranges in urinary concentrations among the exposed and the nonexposed were noticed in both formic acid and methanol.

To make comparison in a more quantitative manner, it is possible to employ the strategy recently developed by this group (Kawai et al. 1992b); here, the sensitivity of the method can be evaluated in two ways. One is to compare the 95% upper limit at 0 ppm exposure with the

Table 5. Estimated levels of formic acid and methanol in urine after exposure at 0 and 200 ppm

Analysis for	Exposure at			
	0 ppm[a]		200 ppm[b]	
	Men	Women	Men	Women
Formic acid (mg/l)	29.4	33.7	38.4	34.0
Methanol (mg/l)	6.1	23.0	29.2	22.3

[a] The 95% upper limit at 0 ppm
[b] The 95% lower limit at 200 ppm

Table 6. Comparison of urinary formic acid and methanol levels as estimated by various authors

Reference	Methanol exposure	Urinary formic acid	Urinary methanol
Liesivuori and Savolainen (1987)	200 ppm[a]	81 mg/g creatinine (43 mg/g creatinine)	–
Heinrich and Angerer (1982)	93 ppm	30 mg/l (33 mg/l)	26 mg/l (13 mg/l)
Ogata and Iwamoto (1990)	120 ppm	123 mg/l (35 mg/l)	36 mg/l (17 mg/l)

Values are those reported by the referred authors, and those in parenthesis are the estimates based on the present study

[a] 200 ppm, the current occupational exposure limit, is selected for comparison by the present authros

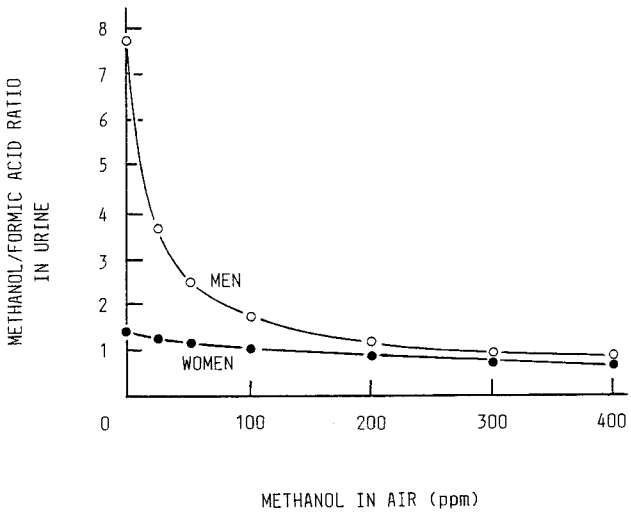

Fig. 6. Dose-dependent changes in formic acid/methanol ratio by sex. Open and solid circles are for men and women, respectively

95% lower limit at LSC-1 ppm (LSC standing for the lowest separation concentration) where the two limit values meet, as depicted schematically in Fig. 4. Alternatively, it is possible to use the upper 95% limit of the background level (or the level in urine samples from nonexposed people) in the place of the 95% upper limit at 0 ppm to find LSC-2 ppm (see Fig. 5). The results are summarized in Table 4. LSC-1 values for formic acid are smaller than those for methanol in women but not in men. Comparison of LSC-2 values cannot be made due to technical difficulty in methanol in women. Another method of evaluation is to compare the power to separate those exposed to methanol at the current occupational exposure limit of 200 ppm (American Conference of Governmental Industrial Hygienists 1991; Deutsche Forschungsgemeinschaft 1991; Japan Association of Industrial Health 1991) from the nonexposed. The worst cases of the exposed and the nonexposed can be represented by the 95% lower limit at 200 ppm and by the 95% upper limit at 0 ppm, respectively. The calculation (Table 5) shows that the separation can be barely achieved for formic acid in both sexes, whereas it is quite possible for methanol in men although barely so in women.

In overall evaluation, therefore, urinary formic acid is no more sensitive than urinary methanol as an indicator of exposure to methanol vapor. This conclusion is in close agreement with the conclusion of Heinrich and Angerer (1982). The fact that analysis for urinary formic acid (30 min/sample) is more time consuming than that for urinary methanol (10 min/sample) is also a practical disadvantage.

Urinary formic acid and methanol concentrations estimated for a given exposure level are different among several authors (Heinrich and Angerer 1982; Liesivuori and Savolainen 1987; Ogata and Iwamoto 1990). The results of the comparison are summarized in Table 6. It appears that the formic acid and methanol concentrations as estimated in the present study results are close to the findings by Heinrich and Angerer (1982) and are smaller than the values reported by Liesivuori and Savolainen (1987) and Ogata and Iwamoto (1990).

The ratio between formic acid and methanol may be worthy of discussion. In the study by Ogata and Iwamoto (1990) on eight male workers exposed to methanol at about 120 ppm, 123.3 ± 168.1 mg/l formic acid and 35.8 ± 41.5 mg/l ethanol (both as AM $\pm$ ASD) were detected in the shift-end urine samples; the ratio of formic acid to methanol on a weight basis was 3.67 and that on a molar basis was 2.56. From the regression lines calculated (Table 3), it was possible to calculate the concentrations of formic acid and methanol in urine after exposure to methanol vapor at a given concentration. When the ratio of formic acid to methanol in urine (on a molar basis) was calculated for men and women separately at various methanol vapor concentrations (Fig. 6), a sharp decrease in the ratio was observed for men as a function of increasing methanol vapor concentration from over 7 at 0 ppm to less than 1 at 400 ppm. The trend was essentially reproducible also for women although the changes were less remarkable (Fig. 6). The changes should be a reflection of the two sources for both urinary formic acid and methanol, one being physiologically present in urine (Table 2) and the other as an increment induced by methanol exposure. From the parameters of the regression lines (Table 3), the ratio should approach 0.52 for men and 0.37 for women after exposure to methanol vapor at an infinite concentration.

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