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# Uptake and Disposition of Inhaled Methanol Vapor in Humans

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**Methanol is a widely used solvent and a potential fuel for motor vehicles. Human kinetic data of methanol are sparse. As a basis for biological exposure monitoring and risk assessment, we studied the inhalation toxicokinetics of methanol vapor in four female and four male human volunteers during light physical exercise (50 W) in an exposure chamber. The relative uptake of methanol was about 50% (range 47–53%). Methanol in blood increased from a background level of about 20 to 116 and 244 μM after 2 h exposure at 0, 100 ppm (131 mg/m$^3$) and 200 ppm (262 mg/m$^3$), respectively. Saliva showed substantially higher levels than blood immediately after exposure. This difference disappeared in a few minutes; thereafter the concentrations and time courses in blood, urine, and saliva were similar, with half times of 1.4, 1.7, and 1.3 h, respectively. The postexposure decrease of methanol in exhaled air was faster, with a half time of 0.8 h. The methanol concentrations were approximately twice as high in all four types of biological samples at 200 compared to 100 ppm. No increase in urinary formic acid was seen in exposed subjects. Our study indicates nonsaturated, dose-proportional kinetics of methanol up to 200 ppm for 2 h. No gender differences were detected. Similar, parallel patterns were seen with regard to the methanol time courses in blood, urine, and saliva, whereas the concentration in exhaled air decreased markedly faster. Thus, apart from blood and urine, saliva also seems suitable for biomonitoring of methanol exposure.**

*Key Words:* **methanol; inhalation; toxicokinetics; human; biomonitoring; gender.**

Methanol is a high-production-volume chemical that is widely used both as an industrial solvent and in consumer products. Methanol is important as an intermediate for other chemicals and as a gasoline additive. Methanol has also been suggested as an alternative fuel for motor vehicles to reduce pollution in the urban air. Such use would increase exposure to methanol vapors of workers in specific areas as well as the general population. Methanol is also a natural ingredient of various foods such as fresh fruits, fruit juices, certain vegetables, and the artificial sweetener aspartame (Kavet and Nauss, 1990; Lindinger *et al.*, 1997; Taucher *et al.*, 1995).

Methanol is easily absorbed via both inhalation and ingestion (Liesivuori and Savolainen, 1991), whereas inhalation and skin absorption are the most important entry routes of methanol in occupational exposure. The substance is either excreted unchanged in urine, in exhaled air, and in saliva, or metabolized. The major pathway is oxidation by alcohol dehydrogenase to formaldehyde and further metabolism to formic acid by formaldehyde dehydrogenase (Eells *et al.*, 1981). Formic acid may cause metabolic acidosis, visual impairment, blindness, and death (Reese and Kimbrough, 1993). The most common health effect of long-term exposure to low levels of methanol vapor is CNS and ocular effects. Chronic occupational exposure to methanol vapor concentrations of 365–3080 ppm has resulted in headache, dizziness, nausea, and blurred vision (IPCS, 1997).

The aim of the study was to investigate the uptake and disposition of inhaled methanol vapors. In addition, we studied possible gender differences in toxicokinetics and the suitability of saliva for biomonitoring. The highest exposure condition of 200 ppm of methanol was chosen considering the safety of the volunteers and as being representative of the upper end of likely exposure levels at work. It also corresponds to the Swedish occupational exposure limit (SWEA, 2000).

## MATERIAL AND METHODS

***Subjects.*** Four females and four males participated in the study. The exposures were carried out after a medical examination including a health questionnaire, a general physical examination, and standard clinical blood and urine analyses. The subjects were included in the study only if considered healthy. The following additional inclusion criteria were used: age between 20 and 50, nonsmoker, no history of allergy or chronic disease, no occupational exposure to organic solvents, and not pregnant, as confirmed by pregnancy test (Event test® strip hCG, Boehringer Mannheim, Italy) immediately before the exposure. To reduce the variability in background methanol, the participants

The authors certify that all research involving human subjects was done under full compliance with all government policies and the Helsinki Declaration.

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were instructed to refrain from alcoholic beverages, drugs, and fruits at least 48 h before and throughout the experiment. The volunteers were informed orally and in writing about the design of the study, possible hazards, and their freedom to discontinue whenever they wanted. The regional Ethics Committee at the Karolinska Institutet, Stockholm, approved the study.

***Chemicals.*** Methanol (chemical purity HPLC, <0.05% $H_2O$) was purchased from Fluka Chemie AG, Buchs, Switzerland. Acetonitrile (isocratic grade for liquid chromatography) and potassium dihydrogenphosphate (puris) were purchased from Merck, Darmstadt, Germany. Sodium phosphate (anhydrous, reagent grade) was from Sigma, USA. Formate dehydrogenase, iodonitrotetrazolium chloride (INT), β-nicotinamide adenine dinucleotide (NAD), and diaphorase buffer were all purchased from Sigma, Steinhem, Germany. Formic acid (pro analysis) was purchased from Merck, Darmstadt, Germany.

***Exposure conditions and experimental design.*** The exposures were carried out inside a 20-$m^3$ stainless steel exposure chamber with 18 to 20 air changes per hour. A high-performance liquid chromatography (HPLC) pump (Gilson 302, France) transferred the solvent to a preheated glass tube (90°C), where the solvent was vaporized. The completely vaporized solvent followed the influent air stream into the exposure chamber. Temperature and humidity were continuously recorded (Vaisala HMP 36, ETM, Sweden) and logged on a personal computer in 1-min intervals. Two subjects at a time were exposed at three different times to 100 ppm (131 mg/$m^3$) or 200 ppm (262 mg/$m^3$) methanol or to clean air for 2 h. The subjects were exposed in different exposure orders, and exposure sessions were separated by at least 2 weeks. During exposure the volunteers performed light physical exercise at a workload of 50 W on a bicycle ergometer (Monark Ergomedic 829E, Sweden). Heart rate was recorded by automatic electrocardiographic (ECG) telemetry (Polar PE 3000, Finland). Physical data, including pedaling frequency, actual workload, and heart rate, were continuously recorded in 1-min intervals on a personal computer.

*Sampling and Methanol Analyses*

***Chamber air.*** The concentration of methanol in chamber air was analyzed at 5-min intervals during exposure. This procedure is described in detail by Nihlén *et al.* (1998a). The analyses were performed using a wide-bore capillary column (Poraplot U, 10 m, 0.32 i.d., 2 µm, Chrompack). Nitrogen was used as a carrier gas at a column pressure of 10.6 psig. The temperatures of the oven and the detector were 140°C and 250°C, respectively. The retention time of methanol was 1.2 min.

***Exhaled air.*** Mixed exhaled air was collected once before the exposure, four times during (every 30 min), and eight times after the exposure (at 2.2, 2.5, 3.0, 3.6, 4.1, 5.1, 6.1, and 23 h from the onset of exposure). The procedure is described in detail in Nihlén *et al.* (1998a). To achieve higher analytical sensitivity, exhaled air after the exposure was adsorbed on stainless steel adsorption tubes filled with Carbosieve SIII (60/80 Mesh) (Supelco). About 10 l of mixed exhaled air was collected during 1 min by means of an air pump (Aircheck 224-PCXR8, SKC). Duplicate samples were collected and analyzed in the afternoon after the end of exposure. Samples were desorbed (Perkin Elmer Automated Thermal Desorption system ATD-400) and analyzed using the same GC system as described previously (Nihlén *et al.*, 1998a). The thermal desorption temperature settings were: desorption oven 300°C, desorption time 2 min, valve 200°C, trap low –30°C, high 300°C hold 2.0 min, line 150°C. The pressure was 9.6 psi, the desorption flow 55 ml/min, and outlet split 9.8 ml/min. A Chrompack capillary column (Poraplot U, i.d. 0.32 mm, coating 10 µm) was used in the GC analysis. Nitrogen was used as carrier gas at a flow rate of 2 ml/min. The injector and detector temperatures were 200°C and 250°C, respectively. The column temperature was kept at 45°C for 4 min initially and then raised to 110°C by 5°C/min, held for 2 min, and finally heated by 10°C/min to 180°, and held for 2 min.

***Blood, urine and saliva.*** Venous blood (10 ml) was collected from the brachial vein in heparinised tubes (Venoject VT-100H) prior to exposure. Preexposure venous blood samples were used to confirm the background level of methanol and to prepare analytical calibration standards.

Capillary blood was sampled for analysis of methanol during exposure at 15, 30, 60, 90, and 118 min, (averages, actual sampling times differed slightly between individuals) and after exposure at 2.2, 2.5, 2.9, 3.5, 4.0, 5.0, 6.0, 9.0, 13.0, and 23.0 h from the onset of exposure. Prior to capillary blood sampling, the volunteer immersed the hand in warm water (38–40°C) for 1 min. Blood was collected in two 100-µl heparinized glass capillaries (Kebo AB, Sweden) and immediately transferred to a 20-ml glass headspace vial. The vial was immediately sealed with a Teflon-lined rubber septum and analyzed by head-space GC the same day.

For collection of saliva, two cigarette filters (cellulose acetate, diameter 8 mm, length 20 mm, Austria Tabak Scandinavia AB) were kept for 1 min in the lower jaw outside the teeth. Prior to use, it was assumed that the filters did not contain methanol or other substances disturbing the GC analyses. Approximately, 0.5 ml saliva was sampled. Due to the high water:air partition coefficient of methanol (3330, Kaneko *et al.*, 1994), variations in saliva volume has little influence on the head-space concentration. The filters were transferred to a 20-ml glass headspace vial. The vial was immediately sealed with a Teflon-lined rubber septum and analyzed by head-space GC the same day. Saliva was sampled at the same time points as capillary blood samples after exposure.

Urine was sampled prior to exposure start and at approximately 2.1, 3.9, 5.9, 9.0, 13.0, and 23.0 h. The volunteers were instructed to completely void the bladder on each occasion. Urine was collected in 500-ml glass bottles, which were immediately capped with polyethylene screw caps. Samples were processed within 5 min and analyzed by head-space GC later the same day, except samples taken at home at 9, 13 and 22 h, which were processed the following morning. Further, pH and ammonia in urine were measured as soon as possible the day of sampling. Fractions of urine samples were acidified and stored in glass vials at –20°C for later analysis of calcium and creatinine. Fractions of urine samples were also stored at –20°C for later analysis of formic acid.

Methanol in blood was analyzed by head-space GC using a Carlo Erba Fractovap 2350 GC equipped with an head-space sampler (Hewlett Packard 7694), a wide-bore capillary column (PoraPLOT Q, i.d. 0.53, coating 20 µm, Chrompack), and a flame ionization detector. Prior to injection the vials were thermo stated at 45°C for 15 min. The injector temperature was maintained at 175°C, and the detector at 175°C. The column was kept isothermal at 80°C. Nitrogen was used as carrier gas at a column pressure of 1.1 kg/$cm^2$. The retention time was 3.8 min for methanol. The detection limit was about 0.3 µM.

The GC analyses of methanol in urine and saliva were carried out on a Perkin Elmer 8700 equipped with auto sampler, AS-101, and flame ionization detector. A wide-bore CP-sil 8 50 m (i.d. 0.53, coating 2 µm, Chrompack) capillary column was used, with nitrogen at a pressure of 11.0 psi as carrier gas. The column was kept isothermal at 120°C. The retention time was 2.5 min for methanol. The detection limit for urine and saliva was 0.1 µM.

Perkin Elmer Turbochrom (v. 4.1) was used for peak identification and integration in the blood, urine, and saliva analyses. The method errors, determined from 10 identically prepared samples of each medium spiked with methanol, were generally less than 3%.

***Other chemical analyses.*** Analysis of formic acid was performed using an enzymatic method by Dow and Green (2000) with minor modifications. Urine (100 µl) was mixed with 100 ml of acetonitrile and centrifuged at 1600 × *g* for 10 min. A total of 50 µl of the supernatant was added to 1.25 ml buffered NAD-diaphorase-INT, followed by the addition of 30 µl of formate dehydrogenase (5 U/ml). The samples were mixed and left at room temperature for 15 min, followed by centrifugation for 1 min at 10 000 × *g*. The absorbance was read at 500 nm using a spectrophotometer (UV-1601 Bio spec, Shimadzu). The method error, as estimated from 10 spiked samples, was 3.4% at concentration of 35 mg/l.

The amount of ammonia in the urine samples was determined by the Enzymatic BioAnalysis Urea/Ammonia test from Boehringer Mannheim (Germany). The principle is: in the presence of glutamate dehydrogenase and reduced NADH, ammonia reacts with 2-oxoglutarate to L-glutamate, whereby

NADH is oxidized. NADH was determined by means of its light absorbance at 340 nm (spectrophotometer, UV-1601 Bio spec, Shimadzu). The detection limit for ammonia was 0.08 mg/l.

Calcium and creatinine in urine were analyzed by standard clinical methods at the Department of Clinical Chemistry, Karolinska Hospital, Solna, Sweden. The pH in urine was measured using a standard pH meter (pHM62, Radiometer, Copenhagen). The pH meter was calibrated each day with two standards (pH 4.0 and pH 7.0).

***Symptom ratings.*** The subjects rated the level of perceived discomfort immediately before, during (10, 50, 80, and 104 min), and after (126 and 210 min) each exposure session. Ten questions were answered, related to irritative symptoms (eyes, nose, and throat or airways), the central nervous system (headache, fatigue, nausea, dizziness, feeling of intoxication), difficulty in breathing, and smell of solvent. The ratings were performed using a 100-mm visual analogue scale (Kjellberg *et al.*, 1988) graded from "not at all" (corresponding to 0 mm) through "hardly," "slightly," "fairly," "much," to "almost unbearable" (100 mm). The same questionnaire has been used in several chamber inhalation studies performed with organic solvent vapors in our laboratory (see for example, Ernstgård *et al.*, 1999, 2002; Järnberg *et al.*, 1996; Nihlén *et al.*, 1998b).

***Calculations.*** Uptake was assumed to occur only via the respiratory tract. Respiratory net uptake during exposure was calculated as the pulmonary ventilation multiplied by the difference in solvent concentration in inhaled and exhaled air.

Time courses of methanol in blood, saliva, urine, and exhaled air were fitted to a linear one-compartment model to obtain toxicokinetic parameters. For blood, the kinetic analysis included values during and after exposure (0–6 h), whereas only postexposure data were used for exhaled air, urine, and saliva (2–6 h). Levels beyond 6 h were in general too close to the background to be meaningful. Individual data were used for blood, breath, and saliva. The urinary data were pooled, since the number of samples per individual were few, and the concentrations highly variable. Hence, data did not allow assessment of the kinetics in urine for each individual. The model was solved using Microsoft Excel Solver (v. 2003) by minimizing the residual sum of squares using a weighting factor of 0.5. The areas under the concentration-time curves (AUCs) were calculated by the trapezoidal method.

Most observations were log normally distributed; therefore statistical calculations (means, confidence intervals, *t*-test etc) were made on log transformed data. The significance level was set at 0.05. Statistical comparisons between females and males with respect to exposure conditions were made using repeated measures ANOVA.

The Wilcoxon signed rank test was used to test for differences in symptom ratings between exposure conditions and ratings during and before exposure. Trend analyses with respect to exposure level were made by using the Friedman test. Microsoft Excel (v. 2003) and StatView (v.5, SAS Institute Inc) were used in the statistical analyses.

## RESULTS

Methanol concentrations in the exposure chamber were in average 98.4 ppm (range 97.2–101.2) and 192.4 ppm (range 183.8–205.3). The average temperature was 18.7°C (SD 0.7), 19.8°C (SD 0.2), and 19.5°C (SD 0.8) and average humidity 39.2% (SD 0.6), 39.1% (SD 0.3), and 38.9% (SD 0.9), after exposure to 0, 100, and 200 ppm, respectively. The fluctuations never exceeded ±8% of the desired concentrations. There were no appreciable differences between the exposure levels with respect to humidity. The average temperature was about 1°C lower at the control condition compared to the methanol exposures. However, this difference is probably of minor importance.

Background levels of methanol were detected in all samples during control exposure: blood range 9–76 μM, saliva 4–76 μM, urine 13–86 μM, and exhaled air 0.0005–0.01 μM. For each individual and time point, the exposure-related methanol concentrations were calculated as the difference between the concentrations measured at methanol exposure and that measured at clean air exposure. No difference between genders was seen with respect to background methanol in blood. Background methanol levels in urine were higher in men than in women (35.9 vs. 21.5 μM, $p = 0.03$ in *t*-test). Similar difference was seen for saliva (39.3 vs. 19.0 μM, $p = 0.008$).

Methanol was rapidly absorbed by inhalation. The relative uptake remained stable throughout the exposure and was approximately 50% at both exposure levels (range 47–53%) (Table 1). The blood methanol concentrations reached 116 (94–144) μM after 2 h exposure at 100 ppm and 244 (228–260) μM at 200 ppm methanol. These levels are consistent with linear, nonsaturated metabolism of methanol.

**TABLE 1**
**Toxicokinetic Parameters of Methanol in Eight Volunteers**

| | 100 ppm | 200 ppm |
|---|---|---|
| Respiratory uptake | | |
| Net uptake (mmol) | 5.9 (5.3–6.6) | 10.1 (8.1–12.5) |
| Relative uptake (% of net uptake) | 51.0 (49.1–52.9) | 49.3 (47.3–51.4) |
| Respiratory excretion | | |
| Exhaled postexposure (μmol)[a] | 56.9 (42.2–76.7) | 94.3 (77.2–115.2) |
| Idem, relative to net uptake (%)[a] | 1.0 (0.8–1.2) | 0.9 (0.6–1.3) |
| Half time in exhaled air (h)[b] | 0.8 (0.7–0.9) | 0.9 (0.8–1.1) |
| Urinary excretion | | |
| Urinary excretion (μmol)[c] | 4.6 (3.8–5.7) | 8.9 (7.2–10.9) |
| Idem, relative to net uptake (%)[c] | 0.8 (0.6–1.0) | 0.9 (0.7–1.2) |
| Half time in urine (h)[b] | 1.7[d] | 1.8[d] |
| Salivary parameters[b] | | |
| Half time in saliva (h) | 1.1 (0.8–1.5) | 1.5 (1.2–1.8) |
| Blood parameters[e] | | |
| Concentration at steady state (μM) | 186.3 (156.9–221.2) | 393.5 (351.8–440.1) |
| Half time in blood (h) | 1.31 (1.1–1.6) | 1.5 (1.3–1.7) |
| Apparent total clearance (l/min) | 0.26 (0.22–0.32) | 0.21 (0.17–0.27) |
| Volume of distribution (l) | 30.0 (23.7–38.6) | 27.1 (21.0–35.0) |
| Idem, relative to body weight (l/kg) | 0.38 (0.31–0.46) | 0.38 (0.31–0.46) |

*Note.* Volunteers were exposed to 100 and 200 ppm for 2 h during light physical exercise at 50 W. Corrections for individual background methanol levels and actual exposure concentration were made prior to the toxicokinetic analyses. Values are geometric means, with 95% confidence intervals in parentheses.

[a] Samples collected at 2–24 h.
[b] Samples collected at 2–6 h.
[c] Samples collected at 0–24 h.
[d] Calculated with all samples from the eight volunteers at three time points.
[e] Samples collected at 0–6 h.

Linear (i.e., dose-proportional) kinetics is also indicated when comparing the AUC (0–6 h) of blood methanol at the different exposure levels (Fig. 1A). Blood methanol increased in a monoexponential fashion during exposure (Fig. 2A). The postexposure decline was also monoexponential, considering the background methanol. According to the toxicokinetic model, the elimination half time in blood was about 1.4 h, and the apparent total clearance 0.2–0.3 l/min. The steady-state level of methanol at continuous exposure to methanol was calculated to be 186 and 394 μM at 100 and 200 ppm, respectively (Table 1), again an indication of linear kinetics.

The postexposure time course of methanol in saliva exhibited a biexponential pattern with a short rapid decline immediately after exposure (Fig. 2B). However, after a few minutes the time course in saliva already closely followed that of blood at both exposure levels (Fig. 3). As with blood, the AUC (2–6 h) of methanol in saliva was proportional to the exposure levels (Fig. 1B). The half times of methanol in saliva were similar after exposure to 100 ppm and 200 ppm (1.1 vs. 1.5 h, ns) and also similar compared to blood (1.3 vs. 1.5 h, ns) (Table 1).

As with saliva, the time course of methanol in urine (Fig. 2C) closely followed that of blood after exposure to 100 ppm (Fig. 3A). At 200 ppm the urine levels appeared to be somewhat higher than in blood and saliva (Fig. 3B). This tendency to a deviation from proportionality was also seen when the AUCs (0–6 h) of methanol in urine were plotted against exposure level (Fig. 1C). However, considering the two-fold difference in exposure level, none of these deviations were statistically significant. The increase (compared to control) in urinary excretion of methanol in 24 h averaged 4.6 μmol at 100 ppm and 8.9 μmol at 200 ppm. This corresponds to less than 1% of the dose absorbed via inhalation (Table 1).

Methanol in exhaled air (Fig. 2D) expressed a biphasic pattern, with a rapid decline the first minutes after exposure, followed by a monoexponential decline before reaching the background level after about 6 h. The half time in exhaled air at 2–6 h of 0.8 h was markedly shorter than in blood (1.4 h, $p < 0.001$), urine (1.7 h, pooled data) and saliva (1.3 h, $p < 0.05$) (Fig. 3). The AUC (2–24 h) in exhaled air increased in proportion to the exposure level (Fig. 1D). Only a small fraction (below 1.5%) of the absorbed dose was excreted via exhalation after exposure, suggesting that nearly the entire absorbed methanol is metabolized.

The excretion of formic acid in urine corresponded to 4.5% (95% CI, 1.5–7.5%) and 2.5% (0.4–4.6%) of the absorbed dose after 100 and 200 ppm exposure to methanol, respectively. The cumulative excretion (0–24 h) averaged 178, 256, and 256 μmol at 0, 100, and 200 ppm of methanol, respectively. Neither the excretion rate nor the cumulative excretion of formic acid was significantly affected by exposure to methanol or by gender (Table 2).

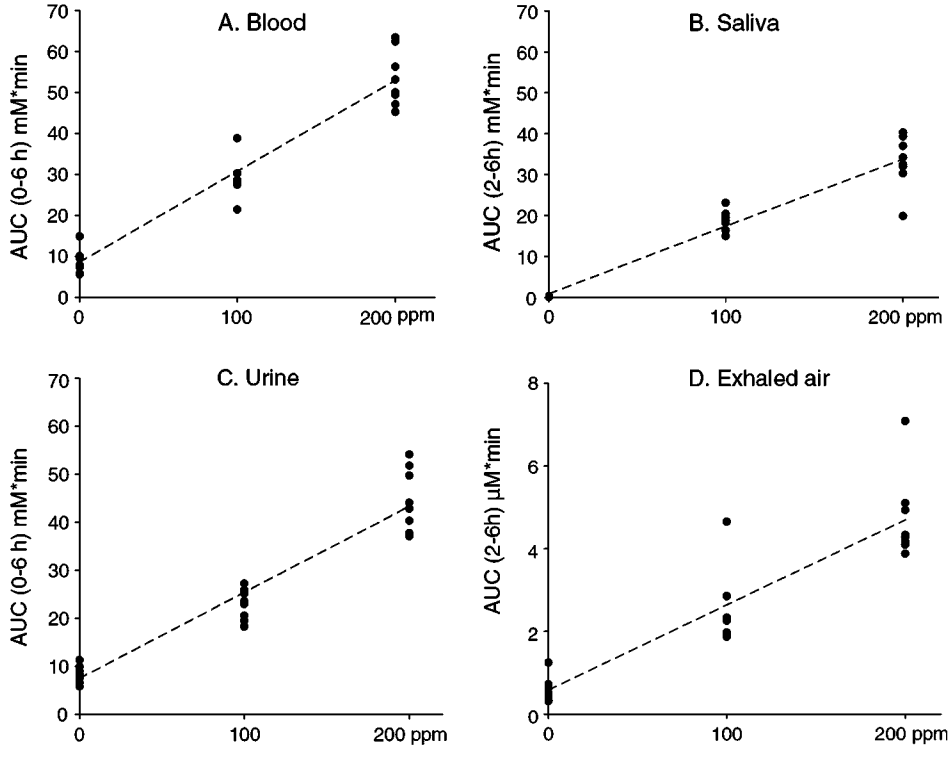


FIG. 1. Area under the concentration-time curve (AUC) of methanol in blood (A), saliva (B), urine (C), and mixed exhaled air (D) from eight volunteers exposed for 2 h to clean air, 100 and 200 ppm methanol during light physical exercise (50W).

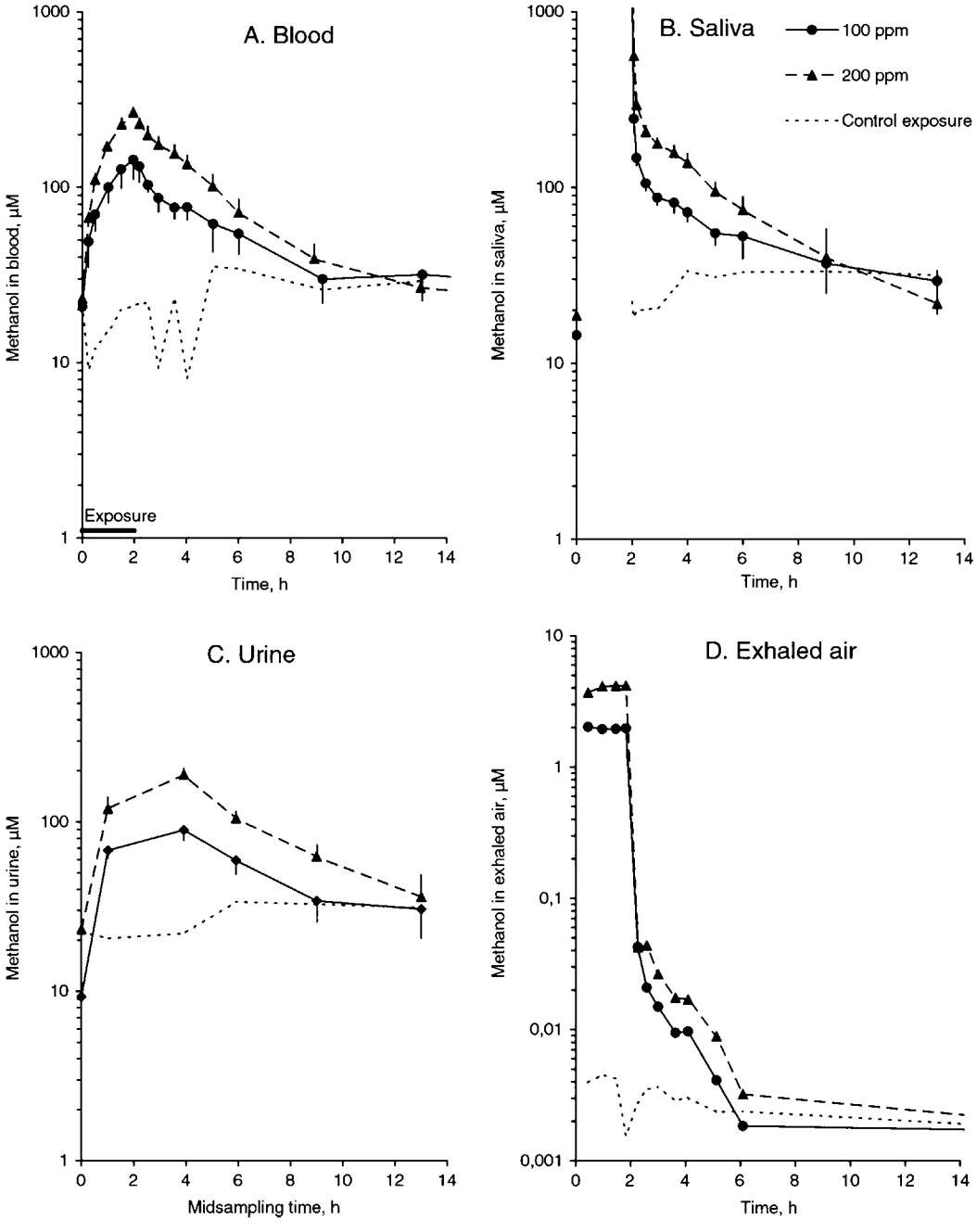


**FIG. 2.** Time courses of methanol in blood (A), saliva (B), urine (C), and expired air (D) in eigh volunteers exposed for 2 h to clean air, 100 and 200 ppm methanol during light physical exercise (50W). Geometric means and upper or lower 95% confidence limits are indicated.

The cumulative excretion of ammonia in urine appeared to be slightly decreased after exposure to methanol; however the effect was only significant at 100 ppm ($p = 0.03$) (Table 2). Independent of exposure conditions, men excreted more ammonia than women ($p = 0.002$).

No effect of methanol exposure was seen on the excretion rate or the cumulative excretion of calcium; however, independent of methanol exposure, the excretion rate was higher in men than in women ($p < 0.0001$). The pH in urine changed significantly over the day ($p < 0.0001$), but no influence of methanol was seen.

No gender differences in any of the toxicokinetic parameters were seen (data not shown).

The average rating of irritation and CNS symptoms during exposure to methanol never exceeded that corresponding to "somewhat" (26 mm). There was no significant difference in

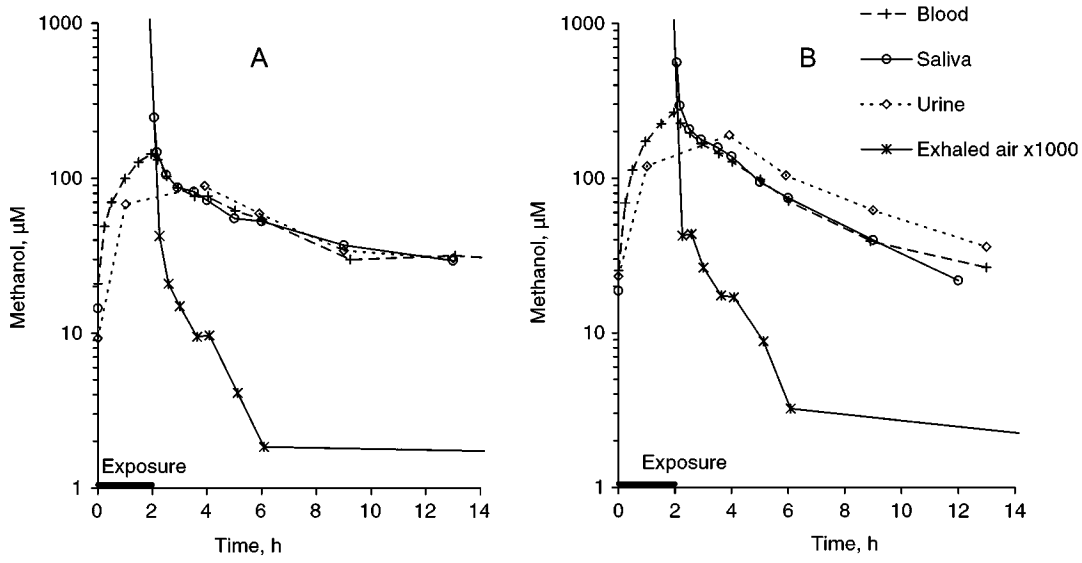


FIG. 3. Comparison of mean methanol levels in blood, saliva, urine, and mixed exhaled air in eight volunteers exposed for 2 h to 100 ppm (A) and 200 ppm (B) of methanol during light physical exercise (50W).

symptoms ratings between methanol exposure and control. However, the women rated significantly higher than the men during exposure to 200 ppm of methanol in three of the symptoms, namely irritation in throat or airways ($p = 0.047$), fatigue ($p = 0.014$), and nausea ($p = 0.045$). At the control condition the women rated significantly higher than men with respect to irritation in the nose ($p = 0.038$).

## DISCUSSION

This study presents the toxicokinetics of methanol in blood, urine, saliva, and exhaled air after controlled inhalation exposure of volunteers to methanol. A few human inhalation kinetic studies of methanol have previously been published (Batterman *et al.*, 1998; d'Alessandro *et al.*, 1994; Osterloh *et al.*, 1996; Sedivec *et al.*, 1981); however, these studies have different limitations in that they have only been carried out with males or have not indicated the gender difference, have only used one exposure level, do not present kinetic data from all the sampling media, and/or do not present kinetic analyses of the data. To our knowledge, our study is the first to investigate possible gender differences in methanol toxicokinetics and also the first to study saliva kinetics in parallel with blood and urine. The extensive sampling in our study makes it possible to compare the time course of methanol in different sampling media. Such knowledge is valuable (e.g., in the development of biological exposure monitoring). Further, we studied the kinetics at two different exposure levels (100 ppm and 200 ppm), as well as during a control exposure to clean air. This design makes it possible to study nonlinear kinetics.

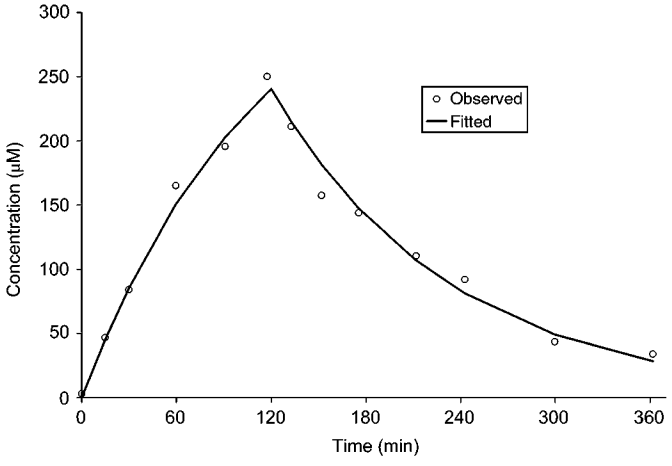


FIG. 4. Concentration of methanol measured in blood in one subject exposed for 2 h to 200 ppm methanol during light physical exercise (50W). Points represent observed values; line shows least-square fit to a one-compartment, linear toxicokinetic model.

In the present study the background level of methanol in blood was 9–76 µM, corresponding to 0.3–2.4 mg/l. This is similar to other studies with reported levels of 0.9 ± 0.6 mg/l (Osterloh *et al.*, 1996), 1.3 ± 0.8 mg/l (Batterman *et al.*, 1998), 1.8 ± 1.2 mg/l (Lee *et al.*, 1992), and 1.3 ± 0.7 mg/l (d'Alessandro *et al.*, 1994). The concentration of methanol in blood reached 228–260 µM (7.3–8.3 mg/l) after 2 h exposure at 200 ppm methanol and a workload of 50 W. The calculated steady-state level ranged from 292 to 477 µM (9.3–15.3 mg/l). This is in agreement with other studies performed at rest; 6.8 ± 1.6 mg/l after 4 h (d'Alessandro *et al.*, 1994), 6.5 ± 2.7 mg/l after 4 h (Osterloh *et al.*, 1996), and 7.0 ± 1.2 mg/l after 6 h (Lee *et al.*, 1992). These comparisons suggest that increased workload (50 W vs. rest) and prolonged exposure

**TABLE 2**
**Excretion Rate and Cumulative Excretion of Formic Acid, Ammonia, Calcium, and pH in Urine in Eight Volunteers**

| | 0 ppm | | 100 ppm | | 200 ppm | |
|---|---|---|---|---|---|---|
| | males | females | males | females | males | females |
| Formic acid[a] | | | | | | |
| Excretion rate (0–2 h) (μmol/min) | 43.3 (12.6–73.9) | 131.2 (39.2–223.3) | 298.5 (92.1–504.8) | 216.2 (0–494.6) | 288.7 (0–585.5) | 357.1 (0–857.8) |
| Cumulative excretion (0–24 h) (mmol) | 130.6 (19.2–242.0) | 223.8 (116.1–331.5) | 298.1 (175.2–421.0) | 103.8 (0–245.5) | 185.2 (21.7–348.9) | 327.4 (0–713.9) |
| Ammonia[b] | | | | | | |
| Excretion rate (0–2 h) (μmol/min) | 37.4 (29.1–48.2) | 17.4 (7.7–39.0) | 34.3 (25.6–45.9) | 16.7 (8.0–34.9) | 31.5 (23.0–43.3) | 20.4 (10.4–39.8) |
| Cumulative excretion (0–24 h) (mmol) | 59.1 (44.8–78.1) | 33.0 (26.2–41.5) | 45.0 (36.5–55.6)[c] | 32.0 (17.1–59.7) | 50.8 (34.9–73.9) | 38.8 (31.3–48.0) |
| Calcium[d] | | | | | | |
| Excretion rate (0–2 h) (μmol/min) | 6.2 (5.4–7.1) | 3.4 (2.9–4.0) | 4.5 (2.2–6.2) | 3.6 (2.8–4.6) | 6.6 (5.6–8.0) | 4.1 (2.9–5.5) |
| Cumulative excretion (0–24 h) (mmol) | 6.1 (4.6–8.1) | 3.2 (2.4–4.4) | 6.6 (4.9–8.8) | 4.9 (4.4–5.5) | 6.6 (4.9–8.8) | 4.9 (4.4–5.5) |
| pH[a] | | | | | | |
| 0–2 h | 5.7 (5.2–6.3) | 5.7 (5.1–6.3) | 5.9 (5.2–6.8) | 5.8 (4.9–6.8) | 6.1 (5.6–6.6) | 6.0 (5.2–7.0) |
| 0–24 h | 5.4 (4.5–6.5) | 5.7 (5.4–6.0) | 5.9 (5.8–6.2) | 5.4 (4.7–6.2) | 5.8 (5.4–6.2) | 5.4 (5.1–5.7) |

*Note.* Volunteers were exposed to 0, 100, or 200 ppm for 2 h during light physical exercise at 50 W. Values are geometric means with 95% confidence intervals in parentheses.

[a]No statistically differences were seen in repeated measures ANOVA.

[b]Men excreted significant more ammonia than women in repeated measures ANOVA ($p = 0.002$).

[c]Men excreted significant less ammonia at 100 ppm compared to 0 ppm ($p = 0.03$).

[d]Men excreted significant more calcium than women in repeated measures ANOVA ($p < 0.0001$).

duration (4 h vs. 2 h) approximately doubles the maximum blood methanol level. Similarly, Lee *et al.* (1992) found a 1.8-fold increase in methanol uptake at intermittent work at 50 W compared to rest. In contrast, the same authors found only a small and statistically nonsignificant increase in blood methanol during exercise.

In our study the estimated half time of methanol in blood was about 1.4 ± 0.3 h. This is in good agreement with a study by Batterman and colleagues (1998), where exposure by inhalation to 800 ppm of methanol during 0.5- to 2-h periods resulted in an estimated half time of 1.44 ± 0.33 h in blood. It is also in agreement with Bouchard and colleagues, who predicted a half-life of 1.7 h in humans by PBPK modeling based on several experimental studies (Bouchard *et al.*, 2001). A longer half-life of methanol in plasma (3.2 ± 2.3 h) was reported by Osterloh *et al.* (1996) (22 subjects inhaled 200 ppm methanol for 4 h). In this study, possible losses and memory effects (absorption of methanol in plastic catheters) during sampling, centrifugation, and storage at –70°C may have occurred, making the half time less reliable.

The AUC of methanol in blood increased in proportion to exposure up to 200 ppm, the highest level tested. The same was seen for AUC of methanol in urine, saliva, and exhaled air (Fig. 1). These results strongly suggest nonsaturated first-order kinetics. Linear kinetics is also supported since the maximum methanol level in blood of 244 μM (7.8 mg/l) is by far lower than the estimated $K_m$ value of 460 mg/l for human and monkey alcohol dehydrogenase *in vitro* (Perkins *et al.*, 1995). Obviously, at higher concentrations such as after an oral overdose of methanol, the metabolism will be saturated, and methanol will disappear from blood following a pattern of zero-order kinetics (Jacobsen *et al.*, 1988).

One may speculate that the lower blood:air (2590, Kaneko *et al.*, 1994) than water:air (3300, Kaneko *et al.*, 1994) partition coefficient of methanol is due to lower affinity to protein than to water. Hence, higher hemoglobin values, as seen in men compared to women, may result in analytical bias with respect to blood measurements and/or differences in the toxicokinetics. We did not measure hemoglobin or hematocrit in the subjects; however, individual standard curves were prepared using blood sampled prior to exposure. No significant differences between individuals were seen in the slope of standard curve, suggesting that influence of hemoglobin is marginal.

In the present study, the background level of methanol in urine was 13–86 μM (0.42–2.75 mg/l). Again, this is in line with previous studies by Sedivec *et al.* (1981) (0.32–2.61 mg/l) and by Batterman *et al.* (1998), who found a baseline concentration of 1.3 ± 0.8 mg/l. The time profiles of methanol in urine and blood were parallel after exposure (Fig. 3) as also found by Batterman *et al.* (1998). The postexposure half times of methanol in urine in our study were 1.7 and 1.8 h, at 100 and 200 ppm, respectively. This is in agreement with the half time of 1.5–2.0 h reported by Sedivec *et al.* (1981) and 1.55 ± 0.67 h, found by Batterman and colleagues (1998).

To our knowledge, our study is the first to measure methanol in saliva in humans. However, drug monitoring by saliva sampling has been used for the past 20 years, and saliva has also been used for sampling of some solvents such as acetone (Ernstgård *et al.*, 2003; Rose *et al.*, 1999), 2-propanol (Ernstgård *et al.*, 2003), and ethanol (Jones, 1979). Methanol is a small, uncharged and hydrophilic molecule with a high water:air partition coefficient of 3330 (Kaneko *et al.*, 1994). Therefore, one may expect rapid equilibration and equal partitioning between saliva and blood. In our study, the time profiles of methanol in saliva and blood were indeed parallel and highly correlated ($r = 0.89$ at 200 ppm) (Fig. 3). Thus, methanol in saliva appears to be suitable for biological exposure monitoring. The major advantages of using saliva are noninvasiveness and easiness of sampling. Cigarette filters are simply put in the mouth for 1 min and then transferred to a head-space vial for GC analysis. Due to the high water:air partition coefficient of methanol, variations in saliva volume have little impact on the head-space concentration. Hence, it is not necessary to use a fixed or well-defined volume. However, saliva sampling should not be performed during or within the first few minutes of an ongoing exposure, since the samples would then be contaminated from ambient air or washout of methanol from the airways.

We found a respiratory uptake of 47–53%. Sedivec *et al.* (1981) used a similar technique (mouth breathing, heated valves) and reported a slightly higher relative uptake of 53–61%; however, this was in resting individuals. The value reported of Batterman *et al.* (1998) of 79% is not comparable, since the first 0.5 l of each breath was excluded from the analysis.

After exit from the exposure chamber, the methanol concentration declined about twice as rapidly in breath as in blood, saliva, and urine (Fig. 3) with a half time of 0.8 h compared to 1.4, 1.3, and 1.7 h, respectively. The initial even faster decline in exhaled air seen during the first few minutes after exposure may be explained by washout from the epithelial lining of the respiratory tract (Johanson, 1986; Johanson and Filser, 1992). A few minutes after exposure to either 100 ppm or 200 ppm the average methanol level was approximately 3000 times lower in breath than in blood. This corresponds very well to the water:air partition coefficient of methanol *in vitro* of 3330. However, this ratio increased to about 20,000 at 4 h after exposure. Similarly, the breath:blood ratio of methanol was about 10000. The reasons for these discrepancies are unknown. One possible explanation that should be further studied is that methanol is metabolized also in the lungs. Breath-alcohol is widely used in testing for drunk driving, as it has been shown that blood and breath ethanol levels are closely parallel (see e.g., Jones and Andersson, 2003). However, the two situations are not comparable, since exposure to methanol vapors results in very low doses, whereas alcohol consumption involves high doses that result in metabolic saturation and slow elimination of ethanol from blood. At present, exhaled air appears to be unsuitable for biological exposure monitoring of methanol.

The excretion of formic acid was seemingly not affected by exposure up to 200 ppm methanol. Similar findings have been reported by other investigators (Chuwers *et al.*, 1995; d'Alessandro *et al.*, 1994; Lee *et al.*, 1992; Osterloh *et al.*, 1996). The absorbed dose of methanol ranged from 8.1 to 12.5 mmol in our subjects. Assuming complete conversion to formic acid and excretion in urine within 24 h, the same amount should be recovered in urine. These amounts cannot be distinguished from the highly variable background excretion of formic acid of 19–332 mmol. In conclusion, formic acid is not a useful biomarker for low-dose methanol exposure.

Metabolic acidosis is characterized by an increase in the excretion of calcium, ammonia, and protons. Urinary calcium excretion was directly and ammonia excretion inversely correlated to urinary formic acid in workers occupationally exposed to methanol or formic acid (Liesivuori and Savolainen, 1987). We saw no consistent changes in calcium, ammonia, or pH and no correlation between the excretion of calcium or ammonia and formic acid after exposure to methanol. The absence of effects on calcium and ammonia and pH in our study may be explained by the low additional internal dose of formic acid.

With the exception of slightly elevate urinary excretion of ammonia and calcium, apparently not related to methanol exposure, we observed no gender differences on methanol toxicokinetics; however, the number of volunteers was low. In contrast, women had a markedly higher exhalation than men of a related alcohol, namely 2-propanol (Ernstgård *et al.*, 2003). Gender differences in ethanol kinetics have been extensively studied (Baraona *et al.*, 2001; Frezza *et al.*, 1990; Kwo *et al.*, 1998). It is well known that women reach higher blood levels of ethanol compared to men after oral intake of equal amounts, expressed per kg body weight, of alcohol (Frezza *et al.*, 1990). The gender difference is partly explained by body build and the remaining difference is due to a smaller gastric metabolism of ethanol in women (Baraona *et al.*, 2001).

We saw no increase in the symptom ratings after exposure to 100 or to 200 ppm of methanol compared to control exposure in our study. This is in agreement with the findings of Muttray and colleagues (2001) who found no differences in symptoms in 12 healthy volunteers exposed to 20 and 200 ppm methanol for 4 h. However, it is noteworthy that women rated significantly higher than men for three symptoms, namely headache, fatigue, and nausea, at 200 ppm methanol. In addition, and in line with our previous experience (Ernstgård *et al.*, 2002), women rated significantly higher than men for irritation in the nose at the control condition.

In conclusion, our study indicates nonsaturated, linear kinetics of methanol following short-term inhalation exposure up to 200 ppm for 2 h. Consistent and parallel patterns were seen with regard to the methanol time courses in blood, urine, and saliva, whereas the concentration in exhaled air decreased markedly faster. Saliva is suggested as a suitable biomarker of

methanol exposure. As expected, the urinary excretion of formic acid did not increase significantly at these exposure levels.

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