Methanol Inhalation: Site and Other Factors Influencing Absorption, and an Inhalation Toxicokinetic Model for the Rat

Robert A. Perkins,[1] Keith W. Ward,[1] and Gary M. Pollack[1,2,3]

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Purpose. This investigation was conducted to identify the site and characteristics of methanol absorption and to develop an inhalation model relating methanol absorption, blood concentration, and elimination.

Methods. Rats were exposed to methanol in chambers that allowed measurement of methanol uptake, ventilation, and blood concentrations; anesthetized rats with a tracheal cannula were examined to determine tracheal concentrations. In separate experiments, methanol-exposed rats received an iv methanol bolus to examine the effect of blood methanol on ventilation and absorption; ventilation also was manipulated by CO_2 or pentobarbital to assess the effect of ventilation rate on methanol absorption. These data were combined to construct a semi-physiologic model of methanol uptake.

Results. Only 1–3% of inhaled methanol reached the trachea, primarily from systemic methanol partitioning into the trachea; blood methanol did not alter methanol absorption. Manipulation of ventilation and application of the pharmacokinetic model indicated that ventilation was less significant than environmental methanol concentration in determining the fraction of inhaled methanol absorbed, although both parameters were important determinants of the total mass absorbed.

Conclusions. These data indicate that methanol uptake is a complex process that depends upon several parameters. Despite these complexities, a relatively simple semi-physiologic model was capable of describing methanol uptake over a wide range of exposure concentrations in the rat.

KEY WORDS: methanol; PBPK models; inhalation absorption; inhalation chambers.

INTRODUCTION

Interspecies extrapolation of xenobiotic disposition is a commonly used method for predicting xenobiotic concentrations in humans based on data generated in laboratory animals. An understanding of the variation between species in the time course of exposure, both systemic and at particular target organs, is founded on an understanding of the dose: the "dose administered" must be known or closely estimated to characterize the toxicokinetics of a given xenobiotic. For gaseous or vapor phase xenobiotics, the mass introduced into the body depends upon the difference between the inhaled concentration (C_{inh}), the exhaled concentration (C_{exh}), and the volume of air that is inhaled (ventilation).

The physiologically-based pharmacokinetic (PBPK) model developed to describe the disposition of inhaled styrene in the rat (1) did not require a measurement of C_{exh}. Accurate predictions were obtained by assuming complete equilibration of styrene vapor with arterial blood in the alveoli. This model does not describe adequately the disposition of polar vapors such as low molecular weight alcohols and ketones (2). While the model predicts that the fraction of an inhaled dose absorbed should be independent of vapor concentration and ventilation rate, the absorbed fraction of a water-soluble vapor varies with inhaled concentration (2).

Methanol has been shown to cause birth defects in rodents when administered via several exposure routes, including inhalation (3–6). Because the public likely will be exposed to increasing amounts of methanol via automotive sources (7), risk to the human conceptus may be significant. Conventional risk analysis for vapor-phase toxicants requires evaluation of absorption and disposition of the compound in animal species in which toxicologic effects have been observed. Extrapolation of toxicant disposition to humans then is attempted; typically, such an extrapolation is based on a PBPK model supported by toxicokinetic data obtained in laboratory animals.

A method was developed in this laboratory to determine ventilation in individual ambulatory rats, with simultaneous analysis of methanol in the airstream and in blood. Experiments in female rats indicated ventilation did not differ statistically with varying methanol exposure concentrations, although ventilation decreased with increasing blood methanol (8). The fraction of methanol absorbed (Φ) was inversely related to the methanol vapor concentration (C_{in}); the ventilation rate, as well as the duration of exposure and/or blood methanol concentration, also affected Φ (9). The present study was undertaken to identify the characteristics of ventilation and methanol absorption that might be important determinants of systemic exposure to vapor-phase methanol.

The first objective of this project was to determine if methanol absorption was limited to the upper respiratory tract (URT). Secondary goals were to determine if blood methanol influenced either the rate of methanol uptake or Φ, and to ascertain the relationship between Φ and ventilation. The final objective of this project was to develop a semi-physiologic pharmacokinetic model for methanol uptake.

MATERIALS AND METHODS

Animals

Female Sprague-Dawley rats (Hilltop Laboratory Animals, Scottdale, PA), 260–430 g, were allowed to acclimate individually in hanging wire cages for one week before experimentation, and were maintained on a 12-hr light/dark cycle with food and

[1] Curriculum in Toxicology, School of Medicine, University of North Carolina, Chapel Hill, North Carolina 27599-7360.

[2] Division of Pharmaceutics, School of Pharmacy, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599-7360.

[3] To whom correspondence should be addressed.

Abbreviations: C_{bld}, blood methanol concentration; C_{exh}, exhaled concentration; C_{in}, chamber inflow methanol concentration; C_{inh}, inhaled concentration; C_{out}, chamber effluent methanol concentration; K_M, Michaelis-Menten constant; K_p, partition coefficient; Φ, absorbed fraction; Q, volume flow; R, rate of transfer of methanol from chamber to animal; $\dot{V}$, minute ventilation; V_d, volume of distribution; V_{max}, maximum elimination rate; V_t, tidal volume; X_B, mass of methanol in blood; X_E, mass of methanol extracted from airstream; X_L, mass of methanol loss in chambers; X_U, mass of methanol taken up by animal.

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water available *ad libitum*. All procedures were approved by the University's Institutional Animal Care and Use Committee. A silicone rubber cannula was implanted in the right jugular vein under ether anesthesia 24 hr before experimentation. Cannula patency was maintained with normal saline containing 20 U/ml preservative-free heparin (Sigma Chemical Co., St. Louis, MO). The cannula was passed through a 20-cm, 3-mm o.d. steel spring tether; the lower end was terminated in a polyethylene cuff that was sutured to the rat.

Exposure Chamber

A rectangular 6.25-L chamber was constructed of glass. The removable polystyrene lid had a one-piece gasket along the perimeter, was tapped with holes for gas, electrical, and instrument connections, and was clamped to the chamber. A polyethylene chimney for the cannula tether was attached to the center of the lid, and was capped by a rubber cork with a small hole for the cannula that could be sealed with high-vacuum grease (Dow-Corning, Midland, MI). A 1.5″ fan (3.5 cfm, Archer, Fort Worth, TX) was covered with metal screening and attached to the lid.

Calibration for Ventilation

Tidal volume (V_t) was evaluated by published methods (10–12). A general description of the method used in this laboratory was presented previously (8). Briefly, the increase in intrachamber pressure (P_m) due to heating and humidification of inspired air is measured and compared to the change in pressure (P_{cal}) produced by injecting a known volume (V_{cal}) of air into the chamber. V_t is calculated as

$$V_t = \frac{V_{cal} \cdot P_m}{P_{cal}} \cdot G_A \quad (1)$$

where G_A, the volume ratio of inhaled gas in the chamber to inhaled gas in the alveoli, was estimated by a published method (10).

Chamber Flow Calibrations

Source air was supplied to the chamber from a compressor. Flow was split, with one portion passed through neat liquid methanol (HPLC grade, Mallinckrodt, Paris, KY) at 0°C. Total flow (Q; 1.1 ± 0.1 L/min) was controlled with rotameter valves (Cole-Parmer, Chicago, IL) and measured with a bubble meter. The flow of air or methanol supply air was adjusted to obtain the desired methanol exposure concentration (± 5%). Chambers and gaskets were allowed to equilibrate with methanol overnight prior to the experiment.

Analysis of Methanol Vapor in Air

The concentration of methanol in air was quantitated by gas chromatography (Shimadzu GC14A, Columbia, MD) with dual injectors, columns (Carbowax, 15 m, 0.54 mm i.d., 1.2 μm film, Alltech Associates, Deerfield, IL), and flame ionization detectors. Gas from the chamber was delivered by a peristaltic pump (Bacon Technical Industries, Concord, MA) through a 4-way rotary valve to an injection valve (VICI, Houston, TX) that released a 1-μl sample to the column. The interconnecting tubing was 0.07″ i.d. stainless steel. The pump, tubing and dead space were calibrated so that approximately 40 volumes were pulled through the system prior to each injection. All components were connected to a microprocessor that controlled sample timing. Calibration of the system was performed by sampling from 2.3-L gas bottles with a minimum of 3 samples at each of 4 concentrations bracketing the expected experimental conditions.

Analysis of Methanol in Blood and Urine

The procedures used to quantitate methanol in biologic samples are described in detail elsewhere (13, 14).

Exposure Regimens and Methods

Individual rats were exposed to methanol vapor for 8 hr at various concentrations as discussed below. Following placement in the exposure chamber, C_{in} and C_{out} were measured at timed intervals (6/hr for the first 2 hr and 2/hr thereafter). Minute ventilation ($\dot{V}$) was determined as the product of V_t and breathing frequency. Determination of $\dot{V}$ required disconnecting the air/methanol supply for 30 sec.

Upon initial exposure, some of the components of the apparatus adsorbed methanol. In addition, as determined in pilot experiments with an empty chamber, there was a slow methanol loss throughout the experiment. These nonspecific losses (including adsorption to the fur and skin as determined in a chamber containing a dead animal) were quantitated and used to calculate the actual mass extracted by the rat.

Data Analysis

The rate of methanol extraction from the chamber airstream may be expressed as

$$\frac{dX_E}{dt} = Q \cdot (C_{in} - C_{out}) - \frac{dX_L}{dt} \quad (2)$$

where X_L represents the sum of nonspecific methanol losses. Integration of Equation 2 over time gives X_E^t, the mass of methanol extracted through time t. Replacing the integral with a summation based on the average concentration difference at the beginning and end of each time period, yields

$$X_E^t = \sum_{i=0}^{t} \left(Q \cdot \left(\frac{C_{in,1} + C_{in,i-1}}{2} - \frac{C_{out,i} + C_{out,i-1}}{2} \right) - \frac{\frac{X_{L,i}}{dt} + \frac{X_{L,i-1}}{dt}}{2} \right) \cdot (t_i - t_{i-1}) \quad (3)$$

The rate of methanol uptake (dX_U/dt) is

$$\frac{dX_U}{dt} = \Phi \cdot \dot{V} \cdot C_{out} \quad (4)$$

The methanol concentration measured in the effluent is assumed to be the same as C_{inh}, i.e., the chamber is a well-stirred reactor as demonstrated previously (9). The uptake rate is integrated over time t to yield the total uptake X_U^t. Uptake was computed using an average C_{out} and $\dot{V}$ as in Equation 4 above, and assuming $\Phi = 1.0$. It should be noted that if Φ equals 1.0,

100% of the inhaled methanol vapor is absorbed and X_U equals X_E. The value of $\dot{V}$ used was based on the volume of air resident in the lungs at full expansion when the inspired air is heated and humidified completely.

To perform a mass balance with X_E, the body burden of methanol at any time t, X_B^t, can be computed from the analysis of the methanol in the blood at time t and the estimated mass of methanol eliminated through time t:

$$X_B^t = C_{bld,t} \cdot V_d + \int_0^t V_{max} \cdot \frac{C_{bld}}{K_M + C_{bld}} \quad (5)$$

where C_{bld} is the instantaneous blood methanol concentration, $C_{bld,t}$ is the concentration at a discrete time, and V_d, V_{max}, and K_M are the apparent volume of distribution, maximum velocity of elimination, and the methanol blood concentration at which velocity is half maximal, respectively. Relevant values of these parameters governing systemic methanol disposition were determined in previous studies (15). Integration of Equation 5 was performed using finite time increments. For determination of mass eliminated, values of C_{bld} represent observed blood concentrations; for intervals over which a blood sample was not obtained, linear interpolation was used between known blood methanol concentrations. X_B will equal X_E if the elimination parameters during inhalation are the same as those following iv or po administration.

The fraction absorbed (Φ) over any incremental time t can be computed as:

$$\Phi = \frac{(X_E^t + X_B^t)/2}{X_U^t} \quad (6)$$

In theory, X_E and X_B are equal and are semi-independent measures; either could have been used alone.

C_{in} and C_{out}, required for computation of the amount extracted from the airstream, were measured at fixed time intervals that seldom corresponded to the exact time of $\dot{V}$ determination or blood sampling. In order to combine data from various animals in each exposure group, X_E, X_U, and X_B were calculated at integer hours using linear interpolation from the two nearest data points. Data were corrected for differences between the actual and nominal exposure concentrations.

Methanol in Tracheal Air

In studies designed to examine the site of methanol absorption, four rats were anesthetized with urethane (1.8 g/kg, i.p.). The right jugular vein was cannulated as described above. The trachea was cleared of surrounding tissue, the isthmus of the thyroid was located, and a section of PE-60 tubing (Clay Adams, Parsippany, NJ) was inserted into the trachea caudal to the larynx (n = 2) or to the back of the nose (n = 2). Cyanoacrylate ester glue was used to fix the PE-60 tubing to the trachea. The mouth also was glued shut to prevent diffusion of methanol into the trachea. The rat was placed in the chamber with both jugular and tracheal cannulae externalized. Animals remained anesthetized during the entire 8-hr exposure to 12,500 ppm methanol. At timed intervals, gas samples were drawn from the PE-60 cannula through duplicate 300-µl plastic vials to separate droplets of aspirated fluid (blood or mucus). At least once each hr, a 25-µl bolus of dichloromethane (DCM) was added to the chamber; DCM is absorbed poorly in the URT (16) and therefore served as a positive control. Blood samples were taken each hr via the jugular cannula and blood methanol concentrations were determined as described above.

Effect of Blood Methanol on Methanol Absorption

To examine the effect of blood methanol on Φ, a bolus of methanol (4 g/kg over 2 min) was administered to rats (n = 2) after 4 hr in the chamber at 5000 ppm. The bolus was calculated to achieve a blood concentration of 5000 mg/L, approximately the concentration observed in the highest exposure group in previous studies in which Φ decreased from 0.84 ± 0.13 at 5000 ppm to 0.60 ± 0.05 at 20,000 ppm (9).

Influence of Ventilation on Methanol Absorption

To examine the relationship between Φ and $\dot{V}$, experiments were designed to monitor Φ while $\dot{V}$ was perturbed. Rats were exposed to 15,000 ppm methanol for 4 hr, at which time $\dot{V}$ was increased by CO_2 (4% of inlet flow; n = 2) or decreased by pentobarbital administration (40 mg/kg; n = 2). Control animals (n = 3) were exposed to methanol alone.

Model Derivation

Development of the toxicokinetic model began with simple mass balance. For the chamber, the relevant mass balance is

$$Q \cdot C_{in} = Q \cdot C_{out} + \frac{dX_L}{dt} + R \quad (7)$$

where R represents the rate at which methanol is transferred to the rat from the chamber control volume. For the rat,

$$R = \Phi \cdot \dot{V} \cdot C_{out} \quad (8)$$

as in Equation 4 above. Combining Equations 7 and 8 and solving for C_{out} yields

$$C_{out} = \frac{Q \cdot C_{in} - \frac{dX_L}{dt}}{Q + \Phi \cdot \dot{V}} \quad (9)$$

Substituting Equation 9 into Equation 8 yields

$$R = \Phi \cdot \dot{V} \cdot \left(\frac{Q \cdot C_{in} - \frac{dX_L}{dt}}{Q + \Phi \cdot \dot{V}} \right) \quad (10)$$

or

$$R = \frac{Q \cdot C_{in} - \frac{dX_L}{dt}}{\frac{Q}{\Phi \cdot \dot{V}} + 1} \quad (11)$$

Converting from mass to concentration with the volume of distribution (V_d), during exposure:

$$\frac{dC_{bld}}{dt} = \frac{1}{V_d} \cdot \frac{Q \cdot C_{in} - \frac{dX_L}{dt}}{\frac{Q}{\Phi \cdot \dot{V}} + 1} - V_{max} \cdot \left(\frac{C_{bld}}{C_{bld} + K_M} \right) \quad (12)$$

Equation 12 is similar to a one compartment pharmacokinetic

model with a constant infusion at a rate equal to mass transfer (17), and can be computed on a spreadsheet program by substituting finite time increments of 0.1 hr for the differential and using the blood methanol concentration from the previous time step for the C_{bld} of the elimination term. Comparative calculations indicated no important advantage in decreasing the time step by a factor of 10.

The kinetic parameters V_d, V_{max}, and K_M for the rat were determined after iv or po administration (15). Of the parameters in the mass transfer term of Equation 12 (C_{in}, Q, X_L, $\dot{V}$, and Φ), the first three were assumed to be known. Since previous work in this laboratory indicated that $\dot{V}$ is a function of blood concentration, $\dot{V}$ was adjusted at each step to account for the variation with blood methanol. In earlier experiments, $\dot{V}$ and blood methanol were evaluated simultaneously for a range of concentrations (8, 9). Those data were analyzed herein by nonlinear least-squares regression (PCNONLIN, version 3.0, Statistical Consultants, Inc., Apex, NC) with the equation

$$\frac{\dot{V}_0 - \dot{V}}{\dot{V}_0} = m \cdot \log_{10}(C_{bld}) + b \qquad (13)$$

or

$$\dot{V} = \dot{V}_0 \cdot (1 - (m \cdot \log_{10}(C_{bld}) + b)) \qquad (14)$$

where $\dot{V}_0$ is the $\dot{V}$ of unexposed rats, and m and b are the slope and y intercept of the straight line. To evaluate Φ as a function of C_{in} and $\dot{V}$, the data (Φ vs. $\dot{V}$ and C_{in} for each hour of all experiments) were pooled. Nonlinear least-squares regression was used to fit the equation

$$\Phi = C_{in}^{a} \cdot \dot{V}_{hr}^{c} \qquad (15)$$

to the data, where C_{in} is the exposure concentration in units of ppm/1000 and $\dot{V}_{hr}$ is the hourly ventilation expressed as L/kg/hr; a and c are empirical constants returned by the regression analysis.

Equation 12 represents the complete semi-physiologic model for methanol disposition during inhalation exposure. However, evaluation of Equation 12 requires values of $\dot{V}$ and Φ that are not constant and which have the potential to vary with each time step. $\dot{V}$ is a function of C_{bld} (Equations 13 and 14) and Φ is a function of both C_{in} and $\dot{V}$ (Equation 15). Thus, Equations 14 and 15 were used in conjunction with Equation 12 to predict methanol blood concentrations during exposure.

RESULTS

Methanol in Tracheal Air

Methanol concentrations entering and leaving the chamber, as well as in tracheal air, are displayed in Figure 1. Only a small fraction of inhaled methanol (1.5% of C_{in}) reached the upper trachea. Similar results were obtained in rats with cannulae pushed to the back of the nose (data not shown). The small amounts of methanol observed in tracheal air may not represent methanol in the inspired airstream, but rather equilibration between systemic methanol and tracheal air. There is rapid distribution of methanol in total body water (14), which would include the tissue and probably the mucus of the URT; these likely are in equilibrium with blood methanol. Blood methanol concentrations divided by the blood:air (3000) or water:air (7000) partition coefficient (K_p) suggest that methanol in tracheal air was derived from a systemic source.

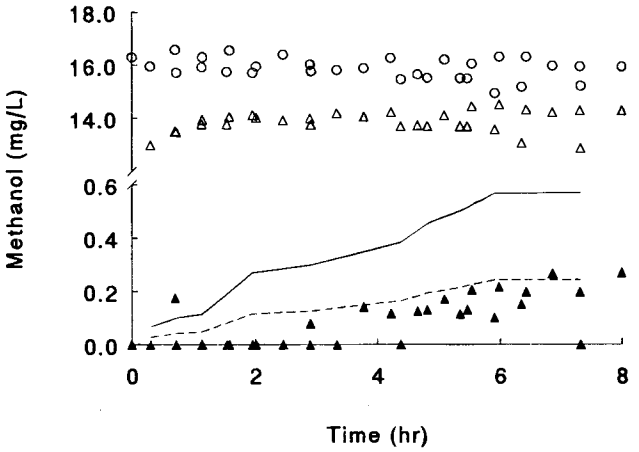

Fig. 1. Methanol vapor concentrations during a 12,500-ppm exposure of rats with a tracheal cannula. Data points are from 2 individual exposures: ○ = C_{in}, chamber influent methanol concentration; Δ = C_{out}, chamber effluent methanol concentration; ▲ = methanol concentration in tracheal cannula; solid line = blood methanol concentration/3000 (the approximate blood: air partitioning of methanol); dashed line = blood methanol concentration/7000 (the approximate water:air partitioning of methanol).

Effect of Blood Methanol on Methanol Absorption

The effect of blood methanol on ventilation and methanol uptake is shown in Figure 2. Following the methanol bolus, X_B increased ~7-fold and $\dot{V}$ decreased (presumably due to

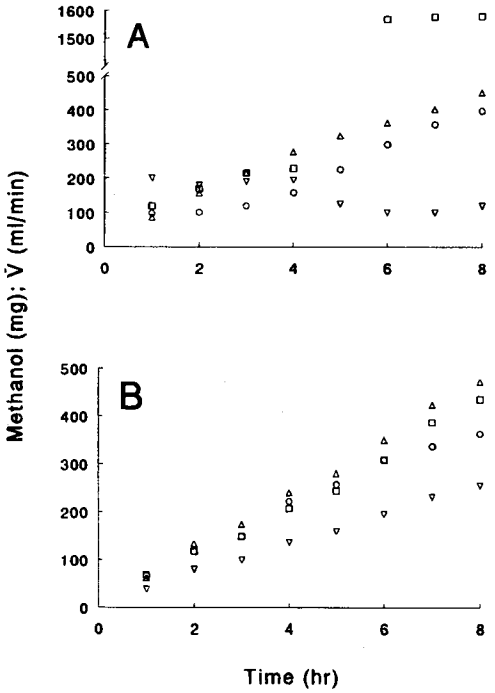

Fig. 2. Methanol uptake during a 5000-ppm exposure (A) in a representative rat receiving an iv bolus of methanol at 4 hr or (B) a representative control. ∇ = $\dot{V}$; ○ = X_E; Δ = X_U; □ = X_B.

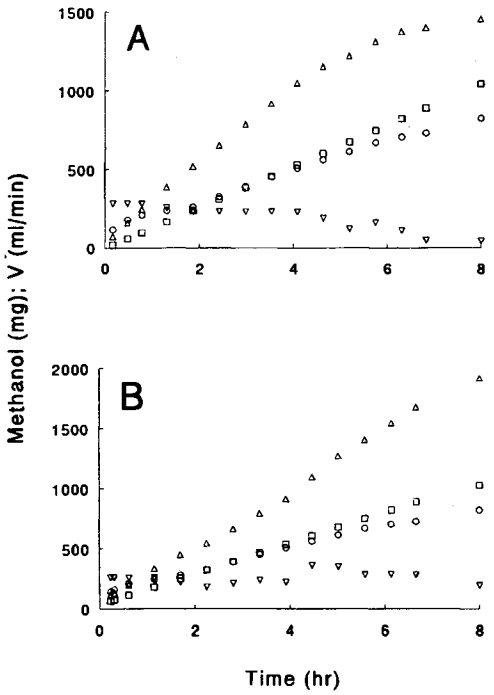

Fig. 3. Influence of alterations in $\dot{V}$ by pentobarbital (A) or CO_2 (B) administration at 4 hr on methanol uptake. Data are from representative rats exposed to 15,000 ppm methanol. ∇ = $\dot{V}$; ○ = X_E; Δ = X_U; □ = X_B.

respiratory depression; 18). However, X_E and X_U continued to increase at the same rate as before the bolus; X_U did not increase relative to X_E post-bolus, indicating that Φ did not change secondary to a significant increase in X_B. Average values of X_E and X_U were similar between rats receiving the methanol bolus and animals exposed to 5000 ppm methanol alone.

Influence of Ventilation on Methanol Absorption

The effect of perturbations in ventilation by pentobarbital anesthesia or CO_2 administration on methanol uptake is displayed in Figure 3. Pentobarbital produced a significant decrease in $\dot{V}$; X_U increased at a slower rate after pentobarbital administration, while the rate of increase in X_B and X_E did not change. In contrast, CO_2 administration resulted in a transient increase in $\dot{V}$ and increased the rate of change in X_U; the relationship between either X_E or X_B and time was not affected. The influence of ventilation on methanol uptake is shown in Figure 4. Total methanol uptake increased with CO_2 exposure and decreased after pentobarbital administration relative to controls. This result was anticipated based on changes in ventilation (Figure 5). Average ventilation remained relatively constant in controls (31.7 L/hr/kg) as well as pentobarbital and CO_2 treated rats during the first 4 hr (34.5 and 31.8 L/hr/kg, respectively). During the second 4 hr of exposure, ventilation in rats receiving pentobarbital decreased to 13.5 L/hr/kg while CO_2 increased ventilation to 42.6 L/hr/kg. CO_2 exposure did not appear to influence Φ; in contrast, pentobarbital anesthesia increased Φ over the last 4 hr of exposure (Figure 5).

Application of Equation 14 across data from all experimental conditions to develop an empirical relationship between $\dot{V}$ and blood methanol yielded m = 0.124 ± 0.030 and b = −0.287 ± 0.080. Similarly, use of Equation 15 to express Φ as a function of inhaled concentration and ventilation yielded a = −0.230 ± 0.041 and c = −0.026 ± 0.020. These results suggest that C_{in} is a more important factor in determining Φ than $\dot{V}$; sensitivity analysis (Table I) indicated that, on a percentage basis, C_{in} is a factor 6 times more important than $\dot{V}$ in determining Φ.

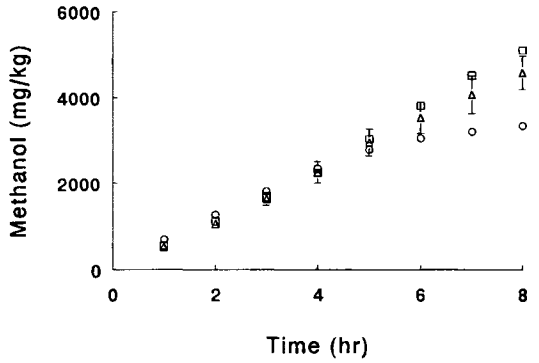

Fig. 4. Average methanol uptake in rats exposed to 15,0000 ppm methanol, computed with Φ = 1.0. ○ = pentobarbital (n = 2); □ = CO_2 (n = 2); Δ = control (n = 3; mean ± SD).

The ability of the semi-physiologic model (Equation 12) with $\dot{V}$ and Φ modulated with time according to the empirical relationships given in Equations 14 and 15, respectively, to predict the methanol body burden during exposure to methanol at various concentrations is shown in Figure 6. At the three highest exposure levels, as well as at 1000 ppm, the model predicted the observed data accurately. At 5000 ppm, model predictions diverged somewhat during the latter stages of exposure.

DISCUSSION

Previous studies have indicated that the likely site of absorption of water-soluble vapors is the URT (19, 20). The duration of these experiments typically was short (< 1 hr); exposures were conducted in highly restrained animals with unidirectional (inhalation only) breathing (21–25). The present results demonstrated URT methanol absorption over an 8-hr exposure in anesthetized rats.

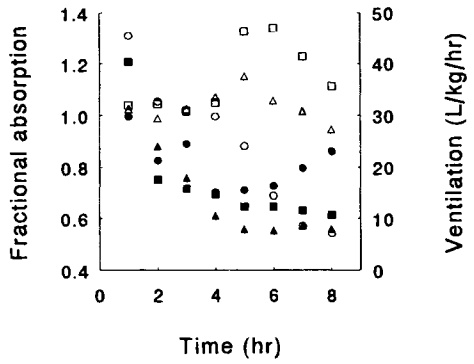

Fig. 5. Time course of Φ (closed symbols) and ventilation (open symbols) in rats exposed to 15,000 ppm methanol. Triangles = untreated, circles = pentobarbital, squares = CO_2.

Table I. Sensitivity Analysis for the Relationship Between ϕ, C_{in}, and $\dot{V}_{hr}$ (Equation 15)

	Sensitivity of Equation 13 to C_{in}		
C_{in} (ppm)	9,000	10,000	11,000
ϕ	0.686	0.677	0.668
% change in ϕ (compared to 10,000 ppm condition)	+1.3%	—	−1.3%
	Sensitivity of Equation 13 to $\dot{V}$		
$\dot{V}$ (L/hr/kg)	28.7	31.9	35.1
ϕ	0.678	0.676	0.677
% change in ϕ (compared to 31.9 L/hr/kg condition)	+0.3%	—	−0.3%

The concentration of methanol measured in tracheal air was consistent with the methanol blood concentration and the water:air K_p. Each tracheal sample (a mixture of inhaled and exhaled air) was collected through two traps, eliminating direct contamination of the sample by blood, mucus or tissue. The K_p that described the data most accurately was water:air rather than blood:air. This result was expected, since the lining of the URT contacted by inhaled/exhaled air is mucus, not blood. A blood:air methanol K_p ~2000 was reported (26); K_p = 2500–3000 was observed in this laboratory (unpublished data). The water:air K_p was estimated to be as low as 2400 (27); experiments conducted during this project indicated this K_p to be 6500–8000 at 25–40°C. The water:air K_p of several alcohols is temperature-dependent (28). The approximate values of K_p used in this study (3000 and 7000) are representative, since the temperature of the trachea was not measured. Despite these limitations, the data are consistent with indirect entry of methanol into tracheal air via the systemic circulation.

The influence of the iv dose of methanol on inhalation uptake was related primarily to changes in ventilation. Fractional absorption of methanol increased slightly after this bolus dose, consistent with earlier observations that Φ increases with decreasing $\dot{V}$ (9) during inhalation exposure to increasing concentrations of methanol. Certainly, Φ did not decrease as would be expected if the capillary blood methanol concentration was an important determinant of methanol absorption. The relationship between changes in Φ and blood methanol concentration with increasing C_{in} or time of exposure therefore appears to be mediated indirectly through methanol-induced changes in ventilation.

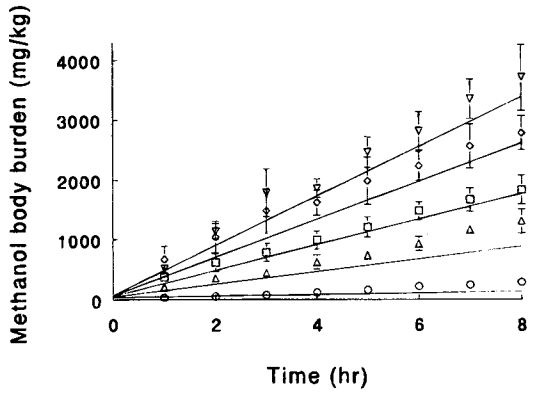

Fig. 6. Result of PBPK modeling of methanol uptake, expressed as body burden of methanol by female Sprague-Dawley rats. Solid lines are the model predictions; symbols represent mean (± SD; n = 3 – 4) data from reference 9. ○ = 1000 ppm; △ = 5000 ppm; □ = 10,000 ppm; ◇ = 15,000 ppm; ▽ = 20,000 ppm.

There was no discernible difference between Φ in control rats and Φ in the rapidly-breathing rats exposed to CO_2. Overall methanol uptake did increase, however, as a consequence of the increase in $\dot{V}$. In contrast, Φ increased in slow-breathing pentobarbital-anesthetized rats; although Φ approached 1.0 by the end of exposure, this may be an overestimate due to a decrease in elimination secondary to anesthesia, since kinetic parameters obtained from unanesthetized rats were used to calculate Φ. Both pentobarbital and urethane decrease cardiac output and decrease blood flow to the liver (29), the primary site of methanol elimination in the rat (18). Methanol is not extracted efficiently by the liver, and the elimination of the alcohol from the systemic circulation should not be related directly to hepatic blood flow. However, other factors associated with decreased organ perfusion (e.g., hepatic oxygenation) may have resulted in decreased metabolism of methanol in these rats.

Previous work in this laboratory (8, 9) and the observations communicated herein support the "wash-in, wash-out" explanation of methanol absorption (25): methanol vapor is extracted completely from the inhaled airstream in the URT; exhaled air, which was scrubbed of methanol upon inhalation, passes over the methanol-loaded URT, producing a large concentration gradient from the URT lumenal surface (mucus and/or tissue) to the exhaled airstream. This concentration gradient favors diffusion of methanol from the URT to air, despite the relatively high water:air K_p, producing a $\Phi < 1.0$. When breathing is very slow, methanol in the URT mucus and tissues has time to diffuse away from the lumen, decreasing the amount of methanol available to the exhaled airstream. Thus, fractional absorption should increase as breathing slows.

The concentration of methanol in blood does not affect methanol absorption *per se,* although it does affect ventilation (9). The apparent absence of a direct effect on absorption is not surprising, since, at the very high blood:air K_p (~ 3000), a 5000-ppm (or C_{in} of 6.55 mg/L) exposure would produce a C_{bld} of ~ 20,000 mg/L at complete equilibration (compared to an observed C_{bld} of 1500 mg/L in rats exposed to 5000 ppm methanol). In the present study, an iv bolus dose of methanol produced a C_{bld} of 5000 mg/L. Even at such a high blood concentration, the mass transfer rate from air to blood did not decrease measurably, leading to an unaffected Φ. Systemic concentrations must approach this equilibration value before Φ would be perturbed.

The semi-physiologic model developed herein demonstrated that the disposition of methanol during inhalation exposure is relatively complex. The total body load at any point in time during exposure is a function of both methanol uptake and elimination. Uptake of methanol from the airstream is dependent on both Φ and $\dot{V}$; $\dot{V}$ is influenced in turn by methanol body load, and Φ is dependent on the exposure concentration and, to a limited extent, on $\dot{V}$. The interdependency of C_{bld} and $\dot{V}$ (and, to a lesser extent, C_{bld} and Φ through the C_{bld}-$\dot{V}$ and

$\dot{V}$-Φ relationships) results in a complicated kinetic-dynamic system required for a comprehensive description of methanol disposition in the rat.

In summary, work in this laboratory to date on development of an inhalation model for methanol indicates that absorption takes place entirely in the URT. Moreover, precise knowledge of the variation of absorption with exposure concentration and time, as well as changes in ventilation with systemic dose, are required to translate ambient methanol vapor concentrations in air into methanol concentrations in blood. Once in the blood, the systemic kinetic parameters (15) enable satisfactory modeling of methanol uptake in the rat. The characteristics of methanol absorption that may influence species differences have been identified herein, and a relatively straight-forward model that accounts for the observed changes in blood methanol concentration, ventilation, and the effect of both factors on absorption at various methanol exposure concentrations has been developed. Work is continuing to determine the degree to which this model generalizes to other species.

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