TOXICOLOGICAL SCIENCES **64,** 169–184 (2001)

A Biologically Based Dynamic Model for Predicting the Disposition of Methanol and Its Metabolites in Animals and Humans

Michèle Bouchard,*,†,[1] Robert C. Brunet,‡ Pierre-Olivier Droz,† and Gaétan Carrier*

**Department of Environmental and Occupational Health, Faculty of Medicine, Université de Montréal, P.O. Box 6128, Main Station, Montréal, Québec, Canada, H3C 3J7; †Institut Universitaire romand de Santé au Travail, rue du Bugnon 19, CH-1005, Lausanne, Switzerland, and ‡Département de Mathématiques et de Statistique and Centre de Recherches Mathématiques, Faculté des arts et des sciences, Université de Montréal, P.O. Box 6128, Main Station, Montréal, Québec, Canada, H3C 3J7*

Received May 10, 2001; accepted August 28, 2001

A multicompartment biologically based dynamic model was developed to describe the time evolution of methanol and its metabolites in the whole body and in accessible biological matrices of rats, monkeys, and humans following different exposure scenarios. The dynamic of intercompartment exchanges was described mathematically by a mass balance differential equation system. The model's conceptual and functional representation was the same for rats, monkeys, and humans, but relevant published data specific to the species of interest served to determine the critical parameters of the kinetics. Simulations provided a close approximation to kinetic data available in the published literature. The average pulmonary absorption fraction of methanol was estimated to be 0.60 in rats, 0.69 in monkeys, and 0.58–0.82 in human volunteers. The corresponding average elimination half-life of absorbed methanol through metabolism to formaldehyde was estimated to be 1.3, 0.7–3.2, and 1.7 h. Saturation of methanol metabolism appeared to occur at a lower exposure in rats than in monkeys and humans. Also, the main species difference in the kinetics was attributed to a metabolism rate constant of whole body formaldehyde to formate estimated to be twice as high in rats as in monkeys. Inversely, in monkeys and in humans, a larger fraction of body burden of formaldehyde is rapidly transferred to a long-term component. The latter represents the formaldehyde that (directly or after oxidation to formate) binds to various endogenous molecules or is taken up by the tetrahydrofolic-acid–dependent one-carbon pathway to become the building block of synthetic pathways. This model can be used to quantitatively relate methanol or its metabolites in biological matrices to the absorbed dose and tissue burden at any point in time in rats, monkeys, and humans for different exposures, thus reducing uncertainties in the dose-response relationship, and animal-to-human and exposure scenario comparisons. The model, adapted to kinetic data in human volunteers exposed acutely to methanol vapors, predicts that 8-h inhalation exposures ranging from 500 to 2000 ppm, without physical activities, are needed to increase concentrations of blood formate and urinary formic acid above mean background values reported by various authors (4.9–10.3 and 6.3–13 mg/liter, respectively). This leaves blood and urinary methanol concentrations as the most sensitive biomarkers of absorbed methanol.

Key Words: **methanol; formaldehyde; formate; toxicokinetics; modeling; animals; humans.**

Methanol is widely used as an industrial solvent and chemical intermediate (Kavet and Nauss, 1990). It has also received serious consideration as an alternative automotive fuel or fuel additive (Health Effects Institute, 1987). Inhalation is a major route of human exposure to methanol in the occupational and general environments although skin exposure can occur in certain industrial settings (Baumann and Angerer, 1979; Downie *et al.*, 1992; Heinrich and Angerer, 1982; Kawai *et al.*, 1991). Exposure to methanol also results from the consumption of certain foodstuffs (fruits, fruit juices, certain vegetables, aspartame sweetener, roasted coffee, honey) and alcoholic beverages (Health Effects Institute, 1987; Jacobsen *et al.*, 1988).

The toxic effects of acute exposures to high methanol doses in humans are well documented (Liesivuori and Savolainen, 1991; Røe, 1982; Tephly and McMartin, 1984; U.S. DHHS, 1993). Neurological effects, such as the initial transient depression of the central nervous system, have generally been reported at blood concentrations of methanol above 6 mmol/l (U.S. DHHS, 1993). However, the severe toxic effects are usually associated with the production and accumulation of formic acid, which causes metabolic acidosis and visual impairment that can lead to blindness and death at blood concentrations of methanol above 31 mmol/l (Røe, 1982; Tephly and McMartin, 1984; U.S. DHHS, 1993).

Although the acute toxic effects of methanol in humans are well documented, little is known about the chronic effects of low exposure doses, which are of interest in view of the potential use of methanol as an engine fuel and current use as a solvent and chemical intermediate. Gestational exposure studies in pregnant rodents (mice and rats) have also shown that high methanol inhalation exposures (5000 or 10,000 ppm

[1] To whom correspondence should be addressed at Département de santé environnementale et santé au travail, Université de Montréal, P.O. Box 6128, Main Station, Montréal, Québec, H3C 3J7, Canada. Fax: (514) 343-2200. E-mail: bouchmic@magellan.umontreal.ca.

and more, 7 h/day during days 6 or 7 to 15 of gestation) can induce birth defects (Bolon *et al.,* 1993; IPCS, 1997; Nelson *et al.,* 1985).

The potential deleterious effects of methanol have prompted extensive research on its uptake and disposition in animals and humans. This has led to the findings that pulmonary absorption of methanol is very rapid and absorption fraction ranges from about 60 to 85% depending on the species (Dorman *et al.,* 1994; Fisher *et al.,* 2000; Horton *et al.,* 1992). Due to the high water solubility of methanol, the distribution of absorbed methanol in the tissues of the body is a function of their relative water content (Sejersted *et al.,* 1983). Animal studies have reported that systemic methanol is eliminated mainly by metabolism (70 to 97% of absorbed dose) and only a small fraction is eliminated as unchanged methanol in urine and in the expired air ($< 3–4\%$) (Dorman *et al.,* 1994; Horton *et al.,* 1992).

Systemic methanol is extensively metabolized by liver alcohol dehydrogenase and catalase-peroxidase enzymes to formaldehyde, which is in turn rapidly oxidized to formic acid by formaldehyde dehydrogenase enzymes (Goodman and Tephly, 1968; Heck *et al.,* 1983; Røe, 1982; Tephly and McMartin, 1984). Under physiological conditions, formic acid dissociates to formate and hydrogen ions. Current evidence indicates that, in rodents, methanol is converted mainly by the catalase-peroxidase system whereas monkeys and humans metabolize methanol mainly through the alcohol dehydrogenase system (Goodman and Tephly, 1968; Tephly and McMartin, 1984). Formaldehyde, as it is highly reactive, forms relatively stable adducts with cellular constituents (Heck *et al.,* 1983; Røe, 1982). It can also enter, directly or after oxidation to formate, the tetrahydrofolic-acid-dependent one-carbon pathway to become the building block of many synthetic pathways (Røe, 1982; Tephly and McMartin, 1984).

The detoxification of formate occurs mainly by a tetrahydrofolate-dependent multistep pathway to carbon dioxide (CO_2) (McMartin *et al.,* 1977; Palese and Tephly, 1975). A small percentage of body formate is also eliminated directly in the urine (Dorman *et al.,* 1994; Horton *et al.,* 1992). Marked species differences in methanol toxicity and metabolism have been reported. Primates and humans appear to be more susceptible to the acute toxicity of methanol than rodents (Tephly and McMartin, 1984). This has been mainly attributed to the slower metabolism and elimination rate of formate in larger species (Tephly and McMartin, 1984).

Based on the available toxicokinetic data of methanol in rats, mice, monkeys, and humans, toxicokinetic processes were described in the past using classic 1 to 3 compartmental models with saturable elimination (Batterman *et al.,* 1998; Damian and Raabe, 1996; Dorman *et al.,* 1994; Nihlén and Droz, 2000; Pollack and Brouwer, 1996; Pollack *et al.,* 1993; Ward *et al.,* 1995; Ward and Pollack, 1996). Physiologically based pharmacokinetic (PBPK) models for methanol in animals and humans were also developed (Fisher *et al.,* 2000; Horton *et al.,* 1992; Perkins *et al.,* 1995; Ward *et al.,* 1997).

Recently, a different type of multicompartment modeling approach has been developed to describe the disposition kinetics of polychlorinated dibenzo-dioxins and furans (PCDD and PCDFs) (Carrier *et al.,* 1995a,b), azinphosmethyl and its alkylphosphate metabolites (Carrier and Brunet, 1999), and methyl mercury and its inorganic metabolites (Carrier *et al.,* 2001a,b). This type of biologically based dynamic model is a refinement of classic compartment models, but is closer to biological processes and enables simulations for a variety of exposure scenarios in different species. This heuristic approach allows essential characteristics of intercompartmental transfer processes to be captured using a minimum of parameters and without the need for extensive knowledge of all the physiological processes. The ultimate goal of this approach is to develop a robust human toxicokinetic model based on human data, thus avoiding as much as possible uncertainties associated with animal to human extrapolations.

The objective of the present study was to develop and validate such a biologically based dynamic model to describe the time evolution of methanol and its metabolites in the whole body, and in accessible biological matrices (blood, urine, and expired air), and allow links to be made between the different compartments. This model is constructed by establishing the overall biological determinants of methanol disposition in animals and humans, taking into account the different time-scales involved in the biological processes. The model parameters specific to the species of interest are then determined from direct fits to the *in vivo* time course data of methanol and its metabolites in blood and excreta (urine and expired air), available in the published literature.

METHOD AND MODEL PRESENTATION

Model Development

A toxicokinetic, biologically based, dynamic model was developed to describe the time evolution of methanol biodisposition in the animal (rat and monkey) and human body. The modeling process can be described in four steps: (1) the conceptual and functional representation of the model, (2) the determination of parameters, (3) the simulation of the kinetic profile, and (4) the validation of the model.

Conceptual and functional representation. The disposition kinetics of methanol and its metabolites following exposure to methanol was modeled using a multicompartment dynamic system, described mathematically by a system of coupled differential equations. The model conceptual and functional representation is depicted in Figure 1. It aims to be sufficiently detailed to describe the available *in vivo* data provided by Horton *et al.* (1992) on the disposition kinetics of methanol and its metabolites in rats. It was then verified that it described equally well the monkey and human kinetic behavior (Dorman *et al.,* 1994; Osterloh *et al.,* 1996; Sedivec *et al.,* 1981).

The whole body (blood and tissues) and the excretory routes (urine and exhaled air) were each represented by a compartment. The whole body loads of methanol, formaldehyde, formate, and unobserved by-products of formaldehyde metabolism were followed. Since methanol distributes quite evenly in the total body water, detailed compartmental representation of body tissue loads was not deemed necessary. Formaldehyde in the whole body was also

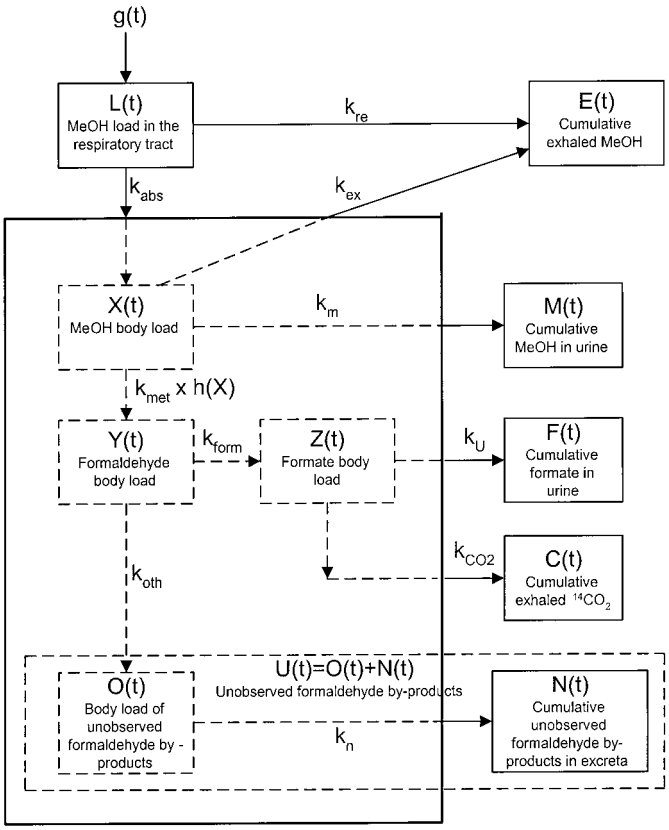

FIG. 1. Conceptual representation of methanol kinetics. Symbols are described in Table 1.

represented as a separate compartment although its metabolism rate is too rapid to allow its quantification (half-life of about 1.5 min according to McMartin *et al.* [1979] and Tephly and McMartin [1984]). It can be shown that, under such fast breakdown, only formaldehyde partitioning between formate and other by-products is relevant to the unfolding of the dynamics.

The respiratory tract was further represented as a separate compartment since it is the route of entry of inhaled methanol. Excretion compartments were the methanol in the exhaled air, the urinary methanol, the urinary formic acid, the CO_2 in the exhaled air, and the excreted unobserved metabolites.

The dynamic of intercompartment exchanges was then described mathematically by a mass balance differential equation system (see Appendix). Essentially, the rates of change in the amounts of methanol and its metabolites in a given compartment were described as the difference between compartment rates of uptake and loss. (Symbols used in the functional representation of the model are presented in Table 1.) Solving numerically the system of differential equations yielded the time courses of methanol and its metabolites in the different compartments.

It should also be mentioned that metabolism was considered to follow Michaelis-Menten kinetics. However, only in the case of methanol metabolism to formaldehyde was a saturation constant introduced since, with the exposure dose range used in the studies on which the model is based, no saturation of formate or CO_2 metabolism was apparent (Dorman *et al.,* 1994; Horton *et al.,* 1992; Osterloh *et al.,* 1996 Sedivec *et al.,* 1981).

Determination of the parameters. Unknown parameters were estimated individually from a statistical best-fit to the experimental data specific to the species of interest, by using the explicit solutions of subsystems of differential equations when possible (see Appendix). A professional edition of a MathCad software was used for this purpose (MathSoft Inc., Cambridge, MA).

Rat parameters. Parameters to be determined were the intercompartment transfer rate coefficients and metabolism rate constants. Data of Horton *et al.* (1992) in male Fischer-344 rats exposed to a single iv dose of 100 mg per kg of body weight of ^{14}C-methanol ($n = 4$) were used to determine the rat parameters. Blood concentration-time profiles (expressed in mg/l) and cumulative urinary excretion time courses of ^{14}C-methanol and ^{14}C-formate (expressed in μmol) were determined by these authors as well as the cumulative exhalation time courses of ^{14}C-methanol and $^{14}CO_2$ (expressed in μmol).

In the current study, for the fitting of experimental data and to determine parameters, all the experimental values were converted to burdens expressed in moles. It was then verified that the mass balance was maintained at all time points. Also, reported blood concentration values were converted to whole body burdens by multiplication by the apparent volume of distribution (V_d). In rats, the apparent volume of distribution of methanol (V_d^{MeOH}) was determined so that the initial experimental concentration of methanol in blood at time t = 5 min, when converted in terms of burden, gives the iv dose (700 μmol) reported by Horton *et al.* (1992).

Monkey parameters. To adapt the model to monkey data, only the values of the intercompartment transfer rates and metabolism constants needed to be modified. Using the same approach as in rats, transfer parameters values of the general model solutions were estimated individually by best-fits, using MathCad, to the available experimental data of Dorman *et al.* (1994). These authors exposed 4 adult female cynomolgus monkeys (*Macaca fascicularis*, 3–5.5 kg) by inhalation to 900 ppm of ^{14}C-methanol for 2 h. Blood concentration-time profiles of ^{14}C-methanol and ^{14}C-formate (expressed in μmol/l) were determined as well as the cumulative urinary excretion of ^{14}C-methanol and ^{14}C-formate 48-h postexposure (expressed in μmol). The time courses of ^{14}C-methanol and $^{14}CO_2$ exhalation rates (μmol/min) were also established. For the determination of the parameter values, the latter rates were converted to cumulative excretion.

The pulmonary ventilation rate of female cynomolgus monkeys used in the model was that reported by Dorman *et al.* (1994), that is on average 33 l/h or 0.56 l/min (equivalent to 0.033 m^3/h or 0.8 m^3/day).

In monkeys, the apparent volume of distribution of methanol was calculated by best-fit of the following equation to the data observed during the constant inhalation built up of methanol blood concentration (B(t)):

$$V_d^{MeOH}(\text{liter}) = \frac{D_{exp}(\mu\text{mol/min}) \times f_{abs}}{k_{e\,lim}(\text{min}^{-1}) \times B(t)(\mu\text{mol/liter})} \times (1 - e^{-k_{e\,lim} \times t})$$

where k_{elim} is the sum of all rates of methanol elimination (metabolism, exhalation, and urinary excretion).

Human parameters. When possible, the constants were determined using the available human data. This includes the pulmonary absorption fraction of methanol, the pulmonary ventilation rate, the apparent volume of distribution of methanol, the metabolism rate constant k_{met} of whole body methanol to formaldehyde, and the transfer rate constant k_m of whole body methanol to urine. The other constant parameters were left as determined in monkeys, which are considered as good surrogates to humans for the study of methanol kinetics.

Pulmonary absorption fraction of methanol used in the model adapted to humans was that reported by Sedivec *et al.* (1981). The human value was nonetheless close to that determined in rats and monkeys. Human pulmonary ventilation rate used in the model was that reported by Sedivec *et al.* (1981), that is on average 10.8 l/min. The apparent volume of distribution of methanol was that reported in the literature, hence, corresponds to the volume of human body fluids (liters), expressed per kilogram of body weight. This value is about the same as that determined using the experimental monkey data.

The constant parameter k_{met} was determined from a best-fit to the blood concentration-time profile of methanol in human volunteers exposed to 200 ppm of methanol vapors for 4 h, as determined by Osterloh *et al.* (1996). The k_m value was determined by adjustment to the data of Sedivec *et al.* (1981) on the urinary excretion time course curves of methanol in volunteers during and following an 8-h inhalation exposure to 300 mg/m^3 of methanol vapors.

TABLE 1
Symbols Used in the Conceptual and Functional Representation of the Model

Symbol	Description
Variables	
g(t)	Pulmonary dose per unit of time that can describe time varying inputs
L(t)	Burden of methanol in the respiratory tract as a function of time
E(t)	Cumulative burden of methanol in the exhaled air as a function of time
X(t)	Whole body burden of methanol as a function of time
Y(t)	Whole body burden of formaldehyde as a function of time
Z(t)	Whole body burden of formate as a function of time
U(t)	Unobserved formaldehyde by-products in the body and excreta as a function of time
O(t)	Whole body burden of unobserved formaldehyde by-products as a function of time
N(t)	Cumulative burden of unobserved formaldehyde by-products in excreta as a function of time
M(t)	Cumulative burden of methanol in urine as a function of time
F(t)	Cumulative burden of formic acid in urine as a function of time
C(t)	Cumulative burden of CO_2 in the exhaled air as a function of time
h(X(t))	The Michaelis-Menten saturation function
Constants	
f_{abs}	Pulmonary absorption fraction of methanol
k_{abs}	Pulmonary absorption rate constant of methanol
k_{re}	Exhalation rate constant of unabsorbed methanol
k_{ex}	Exhalation rate constant of absorbed methanol
k_{met}	Metabolism rate constant of methanol to formaldehyde
K_m	Michaelis-Menten affinity constant for methanol metabolism
k_{fald}	Metabolism rate constant of formaldehyde
k_{form}	Metabolism rate constant of formaldehyde to formate
k_{oth}	Metabolism rate constant of formaldehyde to unobserved metabolites
k_n	Whole body to excreta transfer coefficient of unobserved formaldehyde by-products
k_{CO2}	Whole body to exhaled air transfer coefficient combined with metabolism rate constant of formate to CO_2
k_m	Whole body to urine transfer coefficient of methanol
k_u	Whole body to urine transfer coefficient of formate
V_d^{MeOH}	Apparent volume of distribution of methanol
V_d^{FA}	Apparent volume of distribution of formate

The experimental data of Sedivec *et al.* (1981) on the time evolution of urinary methanol concentrations were converted to cumulative urinary excretion of methanol (in μmol) by considering an average time-dependent fraction of a daily urinary excretion of 1.5 l (Knuiman *et al.*, 1986).

Model simulation. Once the parameters were determined individually by statistical fits to the experimental data, mathematical resolution of the complete model, as represented by the system of differential equations, was performed by the numerical Runge-Kutta method. Model resolution and simulations were also conducted using Mathcad software. This allows prediction of the time evolution of methanol and its metabolites in the different model compartments. In the model, the exposure dose was converted in μmoles for both the iv and inhalation exposures. Thus, whole body burdens and amounts excreted in urine and in the exhaled air are first expressed in μmoles.

In order to simulate the blood concentrations of methanol or formate as a function of time, the amounts in the whole body predicted by the model were simply divided by the respective apparent volume of distribution. For rats and monkeys, the apparent volume of distribution of formate (V_d^{FA}) was estimated using a conservation of mass equation for formate burden, and by a best-fit to the observed time course of experimental blood concentration values of formate. For monkeys, this amounts to 6 times the apparent volume of distribution of methanol. For humans, the same multiple was used.

To simulate the concentration-time profile of methanol in urine, predicted excretion rates ($dM(t)/dt = k_m \times X(t)$, expressed in μmol/min) were divided by the urinary flow rate (l/min). To simulate the concentration-time profile of methanol in the exhaled air, predicted exhalation rates ($dE(t)/dt = k_{re} \times L(t) + k_{ex} \times X(t)$, expressed in μmol/min) were divided by the pulmonary ventilation rate (m^3/min).

Simulations of exposure scenarios, where continuous or intermittent doses are administered through time, were performed by introducing a nonhomogenous term, g(t), describing these time varying inputs (see Appendix). Simulations can also be conducted for different routes of exposure (iv, inhalation).

Model Validation

The model developed using the previously mentioned data was validated using a new set of experimental data. This includes the kinetic time profiles presented in the inhalation studies of Horton *et al.* (1992) in rats and monkeys and Batterman *et al.* (1998) in human volunteers. Also, some human data of Sedivec *et al.* (1981) not used in the development of the model served to validate the model.

Validation using inhalation data of Horton* et al. *(1992) in rats. The model developed using the iv data of Horton *et al.* (1992) in rats was validated with the inhalation data of the same authors, on the blood concentration-time profile of methanol during and following 6-h inhalation exposures to 200, 1200, and 2000 ppm of methanol in male Fischer-344 rats ($n = 4$ per group).

For those simulations, the average pulmonary ventilation rate used was 40 ml/min (equivalent to 0.0021 m^3/h or 0.051 m^3/day) for the 200 ppm dose, 40 ml/min (equivalent to 0.0024 m^3/h or 0.058 m^3/day) for the 1200 ppm dose, and 60 ml/min (equivalent to 0.0033 m^3/h or 0.080 m^3/day) for the 2000 ppm dose to obtain the best-fit to the experimental data as compared to the average

value of 3.04 l/h or 50 ml/min (equivalent to 0.0030 m^3/h or 0.073 m^3/day) reported by Horton *et al.* (1992).

Validation using inhalation data of Horton* et al. *(1992) in monkeys. The model adapted to the monkey data of Dorman *et al.* (1996) was validated using the data of Horton *et al.* (1992) on the blood concentration-time profile of methanol in 3 young adult male rhesus monkeys (*Macaca mulatta*, 5–7 kg) exposed to methanol vapor concentrations of 200, 1200, or 2000 ppm for 6 h. For these simulations, the average pulmonary ventilation rate was that reported by Horton *et al.* (1992), that is 48.9 l/h or 0.81 l/min (equivalent to 0.049 m^3/h or 1.2 m^3/day).

Validation using inhalation data of Sedivec* et al. *(1981) and Batterman* et al. *(1998) in humans. The data of Sedivec *et al.* (1981) on the urinary excretion time course curves of methanol in volunteers during and following 8-h inhalation exposures to 102 and 205 mg/m^3 of methanol vapors were used in the validation process of the model for humans.

The model adapted to human data was also validated using the data of Batterman *et al.* (1998) on the time-dependent disposition of methanol in blood, urine, and breath of volunteers exposed to methanol vapor concentration of 800 ppm for periods of 0.5, 1, and 2 h.

Batterman *et al.* (1998) presented their data as urinary and exhaled concentration-time profiles (expressed in mg/l and ppm, respectively). Although in this article the time courses of methanol cumulative excretion in urine and exhaled air are usually presented to insure mass balance conservation, it was also verified that the model gave a good prediction of the overall concentration-time profiles of methanol in urine and exhaled air (data not shown). To obtain a good fit on both the concentration values and cumulative burdens, a time-dependent fraction of a daily urinary excretion of 2.4 l for the 30 min and 2 h exposures and of 2.7 l for the 1 h exposure had to be considered. It has been reported that the daily personal urine volume may commonly vary from 0.6 l to more than 2.5 l (Knuiman *et al.,* 1986). The average pulmonary ventilation rate used was 11.3, 8.4, and 10.8 l/min for the 30 min, 1 h, and 2 h exposures, respectively, to obtain a best-fit to the exhalation data. These latter rates are in the value range reported by Sedivec *et al.* (1981; average [range]: 10.8 [8.4–13.8] l/min).

RESULTS

Model Developed Using the IV Data of Horton et al. *(1992) in Male Fischer-344 Rats*

Table 2 presents the rat parameter values of the model determined using the data of Horton *et al.* (1992) in male Fischer rats exposed via iv to 100 mg of ^{14}C-labeled methanol per kg of body weight (see Table 1 for the description of symbols). Figures 2 and 3 show that these parameter values allowed to reproduce closely the data presented by Horton *et al.* (1992) on the time courses of blood concentrations of methanol and formate as well as on the cumulative urinary excretion of methanol and formate and the cumulative exhalation of methanol and CO_2.

The estimated average Michaelis-Menten affinity constant value reported in Table 2 and determined using the iv data ($K_{m\text{-}IV}$ of 770 μmol, which represents the body burden of methanol corresponding to half of the maximal velocity for methanol metabolism) shows that when injecting 100 mg/kg of ^{14}C-methanol to Fischer rats (700 μmol), metabolism is not yet saturated. From the product of $K_{m\text{-}IV}$ and k_{met}, an average V_{max} value of 411 μmol/h can be calculated.

The model predicts that methanol elimination from the whole body is quite rapid (mean elimination half-life of 1.3 h) and that, on average, only 0.01% of methanol remains in the unchanged form 18 h following iv injection of 100 mg/kg of ^{14}C-methanol in rats. Peak levels of free formaldehyde in the whole body are reached 0.5 h postdosing, at which time formaldehyde burden represents on average 3.2% of the injected dose. Virtually no free formaldehyde remains in the body 18 h postexposure. The metabolism of methanol to formaldehyde (k_{met}) is predicted to be the rate limiting step in the whole body elimination kinetics of free formaldehyde. Indeed, the biotransformation of formaldehyde to its by-products is estimated to be very rapid ($k_{form} + k_{oth}$ being very large) compared to methanol metabolism to formaldehyde (k_{met}), as apparent when comparing reports of McMartin *et al.* (1979) and Horton *et al.* (1992).

On the other hand, according to model predictions, peak levels of unbound formate in the whole body are reached only 3–3.5 h postexposure where average formate burden represents

TABLE 2

Numerical Values of Constant Parameters Used in the Model Adjusted to Male Fischer-344 rat, Female Cynomolgus Monkey, and Human Data

Constant parameter	Rat value	Monkey value	Human value
Absorption fraction			
f_{abs}	0.60	0.69	0.577[a]
Transfer rate			
k_{met}	0.53	0.96[b]	0.4
k_{form}	14.6	7.2	7.2
k_{oth}	13.0	20.4	20.4
k_{ex}	9.3×10^{-3}	5.1×10^{-3}	5.1×10^{-3}
k_{CO2}	318.6×10^{-3}	813.4×10^{-3}	813.4×10^{-3}
k_m	3.1×10^{-3}	242.1×10^{-6}	2.0×10^{-3}
k_u	11.4×10^{-3}	2.1×10^{-3}	2.1×10^{-3}
Saturation constant			
$K_{m\text{-}IV}$ [c]	770		
$K_{m\text{-}Inh}$ [d]	235		
Volume of distribution			
V_d^{MeOH}	0.92	0.77	0.70
V_d^{FA}	6.4	4.6	4.2

Note. Rat values were determined using the experimental Fischer-344 rat data of Horton *et al.* (1992). Monkey values were determined using the experimental female cynomolgus monkey data of Dorman *et al.* (1994). Human values were kept as in monkeys except for $f_{abs,}$ which is similar to that determined by Sedivec *et al.* (1981); k_{met} was adjusted to the human blood concentration-time profile of methanol of Osterloh *et al.* (1996) and k_m was determined using the human urinary time course data of methanol of Sedivec *et al.* (1981). Transfer rate is given in h^{-1}, saturation constant given in μmol, volume of distribution given as l/kg of body weight.

[a]Used for the simulation of Osterloh *et al.* (1996) and Sedivec *et al.* (1981) studies. For the simulation of the data of Batterman *et al.* (1998), a value ranging from 0.76 to 0.81 was deemed more appropriate, which is in agreement with the mean value of 0.79 reported by those authors.

[b]Used to fit to the female cynomolgus monkey data of Dorman *et al.* (1994). To adapt the model to the data of Horton *et al.* (1992) in male rhesus monkeys, k_{met} value was estimated to be 0.22/h.

[c]Determined using the iv data of Horton *et al.* (1992).

[d]Determined using the inhalation data of Horton *et al.* (1992).

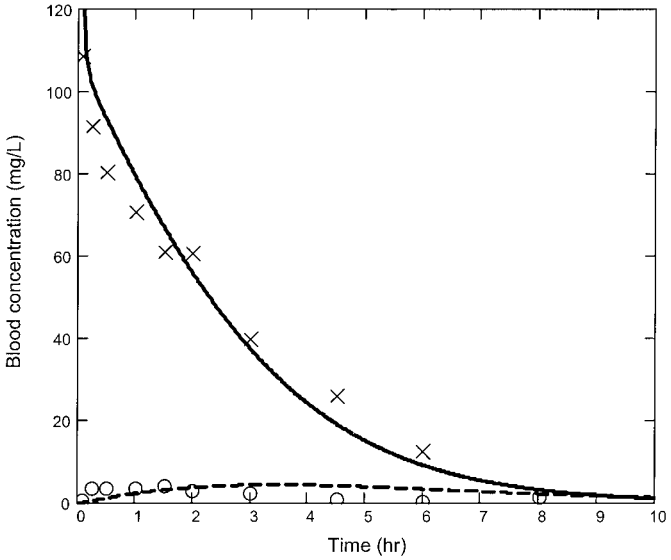

FIG. 2. Model simulations (lines) compared with experimental data of Horton *et al.* (1992) on the concentration-time courses of methanol (crossbars) and formate (circles) in blood over 10 h following a single iv dose of 100 mg/kg of ^{14}C-labeled methanol in male Fischer-344 rats. Each point represents mean value of experimental data ($n = 4$).

20.1% of injected ^{14}C-methanol. Eighteen h postexposure, on average 0.5% of the dose remains in the body as free formate. Initial build-up of unbound formate in the body prior to attrition is dependent on the fact that the metabolism rate constant of formaldehyde to formate (k_{form}) is very rapid (average half-life of about 10 min) compared to the major elimination route of formate, the metabolism rate to CO_2 and subsequent exhalation (k_{CO2}), for which a mean half-life of 2.2 h can be calculated. Since the urinary excretion of formate is negligible compared to CO_2 exhalation, the former contributes only marginally to the whole body time course of formate.

In fact, the model predicts that on average 48.8% of the ^{14}C-methanol iv dose is eliminated as exhaled CO_2 as compared to 1.7% as urinary formate, which is congruent with the experimental results of Horton *et al.* (1992). In comparison, it is estimated from the model that on average 0.8% of the dose is excreted as unchanged methanol in the urine and 2.4% of body methanol is exhaled unchanged again in accordance with the experimental data of Horton *et al.* (1992).

Model Validation Using the Inhalation Data of Horton et al. *(1992) in Fischer-344 Rats*

With the parameter values determined using the iv data of Horton *et al.* (1992), the model was applied to another set of data from the same authors on the blood concentration-time profiles of methanol during and following 6-h inhalation exposures to 200, 1200, and 2000 ppm of methanol in male Fischer-344 rats. It gave a good prediction of the time-course curves for the 2 lowest doses but underestimated the blood concentrations for the 2000 ppm dose (data not shown).

Thus, a new value of the saturation constant K_m was estimated from a statistical best-fit on the blood concentration-time profile data of Horton *et al.* (1992) in male Fischer-344 rats exposed by inhalation to 2000 ppm of methanol vapors for 6 h ($K_{m\text{-}Inh}$) (see Table 2). This $K_{m\text{-}Inh}$ value was about 3 times smaller than that determined with the iv data (on average 235 μmol). Thus, after inhalation exposure to 2000 ppm, saturation of methanol metabolism appears to occur at a lower body burden. With this $K_{m\text{-}Inh}$ value, a V_{max} of 125 μmol/h was calculated. Using this newly determined K_m constant for an

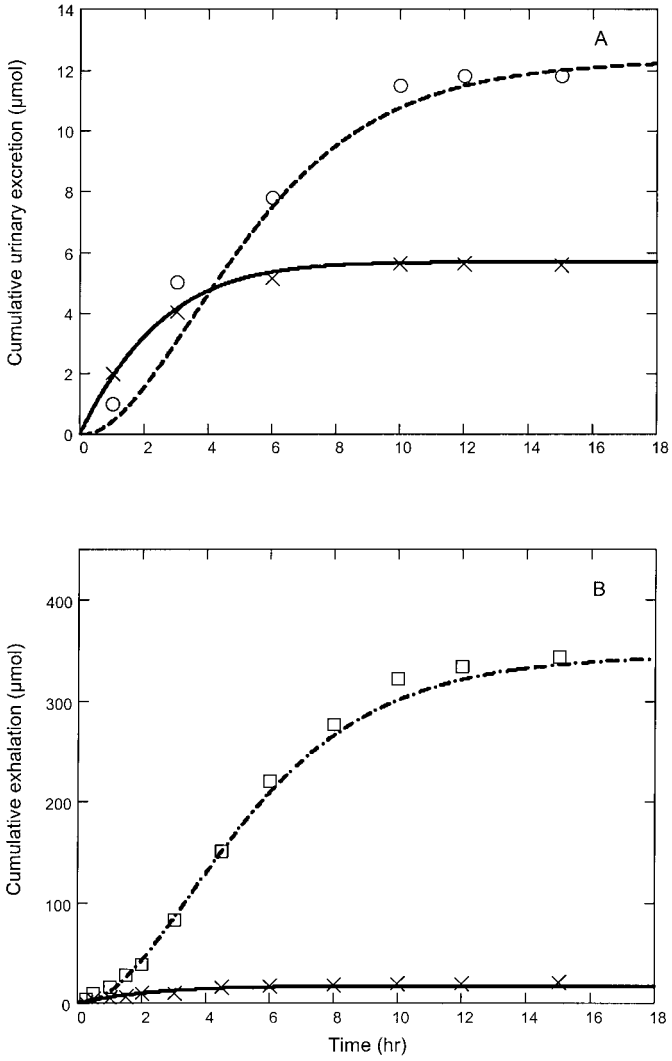

FIG. 3. (A) Model simulations (lines) compared with experimental data of Horton *et al.* (1992) on the cumulative urinary excretion profiles of methanol (crossbars) and formate (circles) over 18 h following a single iv dose of 100 mg/kg of ^{14}C-labeled methanol in male Fischer-344 rats. Each point represents mean value of experimental data ($n = 4$). (B) Model simulations (lines) compared with experimental data of Horton *et al.* (1992) on the cumulative exhalation profiles of methanol (crossbars) and CO_2 (squares) over 18 h following a single iv dose of 100 mg/kg of ^{14}C-labeled methanol in male Fischer-344 rats. Each point represents mean value of experimental data ($n = 4$).

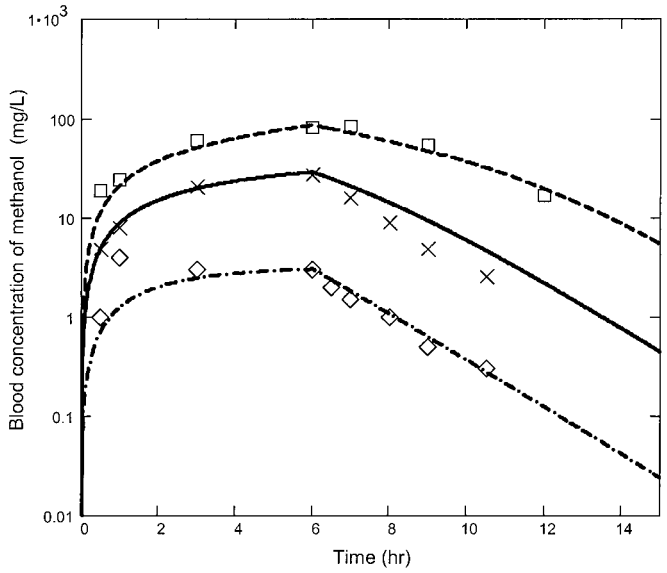

FIG. 4. Model simulations (lines) compared with experimental data of Horton *et al.* (1992) on the time courses of methanol concentrations in blood during and following 6-h inhalation exposures to 200 (diamonds), 1200 (crossbars), and 2000 (squares) ppm of methanol vapors in male Fischer-344 rats. Each point represents mean value of experimental data ($n = 4$).

inhalation exposure, the proposed model provided a close approximation to the data of Horton *et al.* (1992) on the blood concentration-time profiles of methanol in male Fischer-344 rats exposed to vapor concentrations of 200, 1200, and 2000 ppm of methanol for 6 h (Fig. 4).

Model Adapted to Female Cynomolgus Monkey Data of Dorman et al. *(1994)*

Using the conceptual and functional representation of the model established with rat data, the model was adapted to monkeys by adjusting parameters values (see Table 2), through a statistical best-fit, to the data of Dorman *et al.* (1994) in female cynomolgus monkeys exposed by inhalation to methanol vapors. As observed in rats, pulmonary absorption of methanol was estimated to be very rapid (a few minutes) as compared to the metabolism rate constant k_{met} of whole body methanol to formaldehyde. The predicted pulmonary absorption fraction of methanol in monkeys was in the same range as that determined in rats. The estimated monkey constant k_{met} was however 1.8 times higher than in rats. Interestingly, contrary to the rat, according to the data of Dorman *et al.* (1994), no saturation of methanol metabolism was apparent in monkeys even after a 2-h inhalation exposure to 2000 ppm.

Further comparison of monkey and rat parameter values shows that the estimated monkey metabolism rate constant k_{form} of whole body formaldehyde to formate was 2.0 times lower than that of rats. This was also the case for exhalation rate constant k_{ex} of absorbed methanol (1.8 times). The monkey k_{CO2} value, which represents a combined metabolism rate constant of whole body formate to CO_2 and transfer rate constant of CO_2 to the exhaled air, was estimated to be 2.6 times higher than in rats. As observed with the rat data of Horton *et al.* (1992), no saturation of formaldehyde or formate metabolism was apparent from the data of Dorman *et al.* (1994).

It is also noteworthy that the estimated monkey transfer rate constant k_u of whole body formate to urine was 5.4 times lower than in rats and the monkey transfer rate constant k_m of whole body methanol to urine was 12.8 times smaller than that obtained for rats. The estimated monkey apparent volume of distribution of methanol and formate, expressed in liters per kilogram of body weight, were only slightly lower than those of the rats (1.2 and 1.4 times, respectively).

With the parameter values described in Table 2, Figure 5 shows that the model provides a close approximation to the data obtained by Dorman *et al.* (1994) on the blood concentration-time profiles of methanol and formate as well as the time dependent variations in methanol and CO_2 exhalation rates over the 8-h period following the beginning of a 2-h inhalation exposure to 900 ppm of ^{14}C-methanol in adult female cynomolgus monkeys. Although the corresponding detailed urinary excretion profiles of methanol and formate were not depicted by Dorman *et al.* (1994), cumulative excretion of methanol and formate in urine was reported. The model succeeded in reproducing closely these values (0.43 μmol predicted as compared to 0.41 μmol observed on average for urinary methanol, and 1.12 μmol predicted as compared to 1.15 μmol observed on average for urinary formate).

Model Validation Using the Inhalation Data of Male Rhesus Monkeys of Horton et al. *(1992)*

The model, with parameter values adapted as above to cynomolgus monkey data of Dorman *et al.* (1994), was further validated using experimental results of Horton *et al.* (1992) in young male rhesus monkeys exposed for 6 h to vapor concentrations of 1200 and 2000 ppm (These authors also exposed monkeys to 200 ppm but concentration values were too small to provide an accurate prediction.) It was assumed that differences in the kinetics were mainly a result of interstrain differences in the metabolism rate of methanol, k_{met}. With a smaller average k_{met} value of 0.22/h determined by statistical best-fit for larger male rhesus monkeys (5–7 kg) as compared to 0.96/h for smaller female cynomolgus monkeys (3.5–5 kg), the model was able to reproduce the concentration-time course data of methanol in blood of Horton *et al.* (1992) as seen in Figure 6.

It is also interesting to note that, as reported by Horton *et al.* (1992), the model predicts that blood formate concentrations in monkeys will not exceed endogenous background values even for 6-h inhalation exposures to 2000 ppm of methanol vapors (data not shown). This was also observed when simulating the data of Dorman *et al.* (1994) on the blood concentration-time course curve of formate in monkeys exposed to 900 ppm of methanol vapors for 2 h.

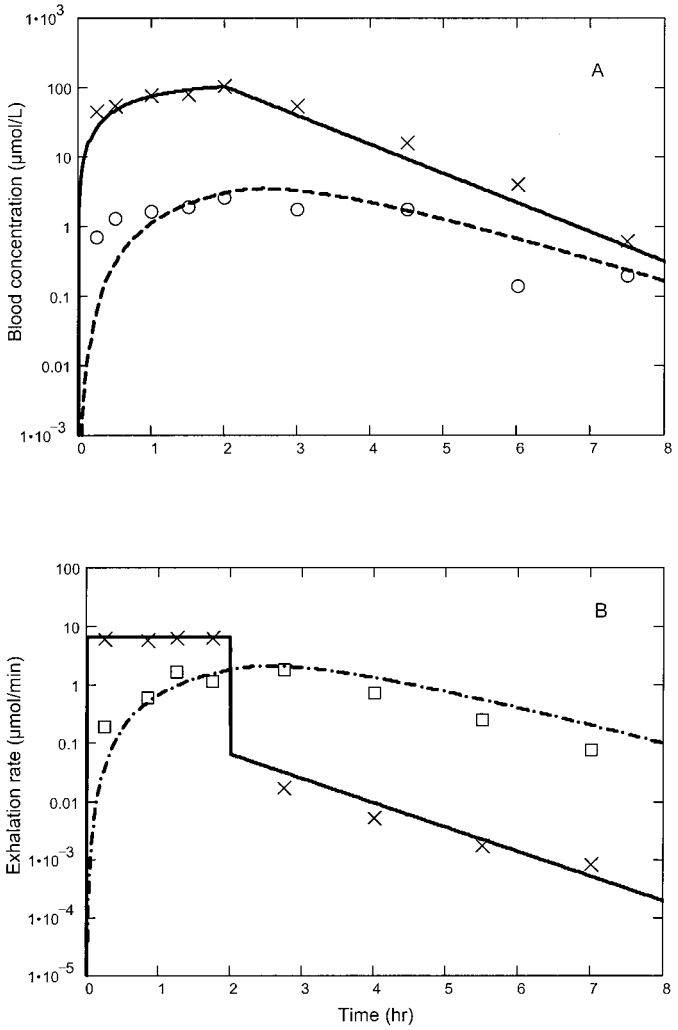

FIG. 5. (A) Model simulations (lines) compared with experimental data of Dorman *et al.* (1994) on the time courses of methanol (crossbars) and formate (circles) concentrations in blood during and following a 2-h inhalation exposure to 900 ppm of ^{14}C-methanol in adult female cynomolgus monkeys (*Macaca fascicularis*). Each point represents mean value of experimental data ($n = 4$). (B) Model simulations (lines) compared with experimental data of Dorman *et al.* (1994) on the time courses of methanol (crossbars) and CO_2 (squares) exhalation rates during and following a 2-h inhalation exposure to 900 ppm of ^{14}C-methanol in adult female cynomolgus monkeys. Each point represents mean value of experimental data ($n = 4$).

Model Adapted to the Human Data of Osterloh et al. *(1996) and Sedivec* et al. *(1981)*

The parameter values estimated by fitting the model to the observed data of Osterloh *et al.* (1996) and Sedivec *et al.* (1981) on the disposition of methanol and its metabolites in humans are presented in Table 2. The estimated value of k_{met} was in the same range as that determined in animals. The k_m value was estimated to be close to that obtained in rats (1.5 times lower) but 8.3 times higher than that of monkeys.

Figure 7 shows that the model simulates correctly the data

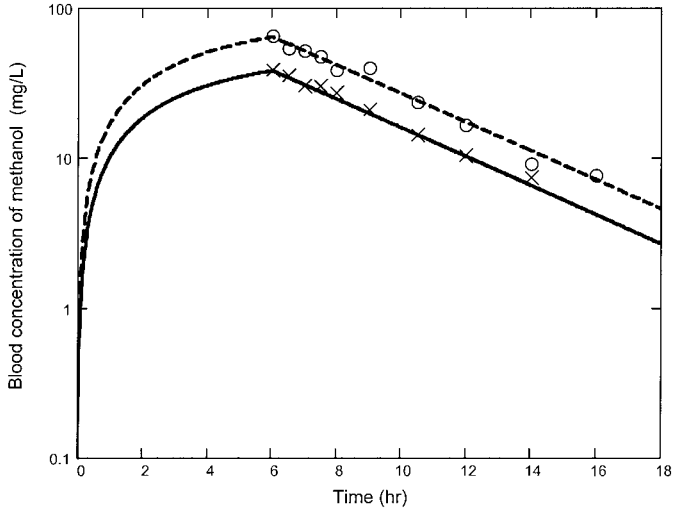

FIG. 6. Model simulations (lines) compared with experimental data of Horton *et al.* (1992) on the time courses of methanol concentrations in blood following 6-h inhalation exposures to 1200 (crossbars) and 2000 (circles) ppm of methanol vapors in young male rhesus monkeys (*Macaca mulatta*). Each point represents mean value of experimental data ($n = 3$).

obtained by Osterloh *et al.* (1996) on the concentration-time course of blood methanol in human volunteers exposed by inhalation to 200 ppm of methanol for 4 h. The model included a constant background whole body methanol burden of 2133 μmol, which corresponds to the mean blood concentration of 1.5 mg/l of methanol measured by Osterloh *et al.* (1996) in control subjects at the end of an 8-h frequent blood sampling

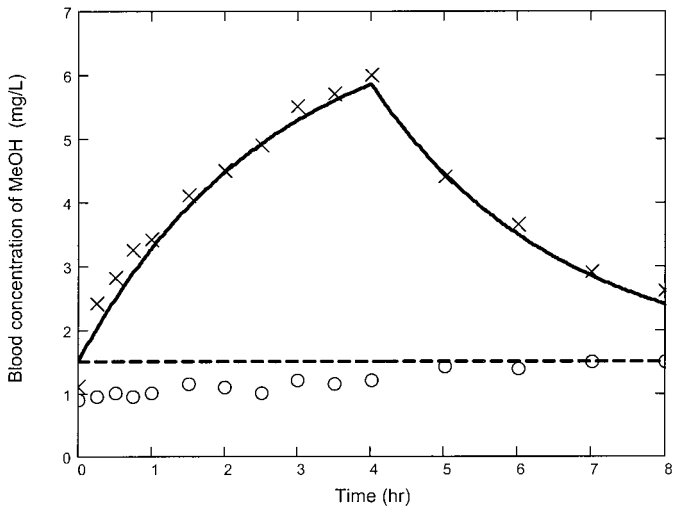

FIG. 7. Model simulations (solid lines) compared with experimental data of Osterloh *et al.* (1996) on the blood concentration-time profile of methanol in human volunteers during and following a 4-h inhalation exposure to 200 ppm of methanol vapors (crossbars). Background blood methanol concentration values considered for model simulations (dashed lines) and experimentally determined by Osterloh *et al.* (1996) over the course of their experimental study (circles) are also represented. Symbols represent mean experimental values from 22 subjects.

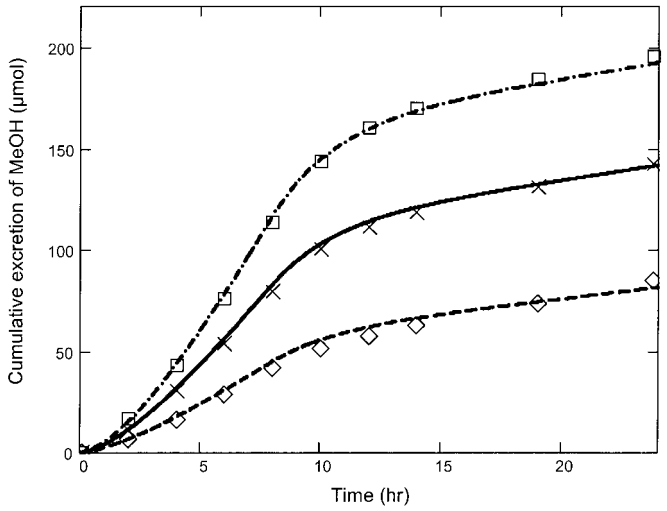

FIG. 8. Model simulations (lines) compared with experimental data of Sedivec *et al.* (1981) on the cumulative urinary excretion time-course of methanol in human volunteers during and following 8-h inhalation exposures to 102 (diamonds), 205 (crossbars), and 300 (squares) mg/m^3 of methanol. Symbols represent mean experimental values from 4 subjects.

period. In accordance with the experimental data of Osterloh *et al.* (1996), the model predicts a log-linear elimination of blood methanol over the 4-h sampling period following exposure (data not shown), indicating the absence of saturation of methanol metabolism for the 4-h inhalation exposure at 200 ppm.

Figure 8 compares model simulations to the time dependent cumulative excretion of methanol in human volunteers during and following 8-h inhalation exposures to 102, 205, and 300 mg/m^3, as determined from the data of Sedivec *et al.* (1981). A mean background whole body burden of methanol of 700 to 1000 μmol depending on the dose (equivalent to a blood concentration of 0.4–0.6 mg/l) was included in the model, which gives predicted baseline urinary concentrations in the same value range as those experimentally observed (0.7 mg/l on average). With these initial conditions, predictions were in close agreement with the experimentally observed data.

Model Validation Using the Inhalation Data of Batterman et al. *(1998) in Human Volunteers*

The model adapted to humans was applied to the data of Batterman *et al.* (1998) in human volunteers exposed to methanol vapor concentrations of 800 ppm for 30 min, 1 h, and 2 h. All the parameter values were kept as determined with the previous human data of Osterloh *et al.* (1996) and Sedivec *et al.* (1981) except for the pulmonary retention. Indeed, for a proper simulation of the experimental data, the average pulmonary absorption fraction had to be 0.76, 0.82, and 0.81 for the 30 min, 1, and 2 h exposures, respectively, which is congruent with the mean value of 0.79 reported by Batterman *et al.* (1998). With these values, Figures 9–11 show that simulations were in close agreement with the experimentally determined concentration-time profiles of methanol in blood, as well as the time evolution of methanol cumulative excretion in urine and in the exhaled air for the various exposure scenarios. A good fit was obtained even when neglecting background methanol in the whole body. Regarding Figure 11, the exhalation rate is almost constant during the exposure, which explains the plateau for the cumulative exhalation.

Prediction of the Time Course Curves of Methanol and Formate Concentrations in Blood and Urine during a 5-Day Continuous Exposure to Methanol Vapor Concentrations of 200 ppm in Humans

The model can also be used to predict the time-dependent variations of methanol and formate concentrations in human

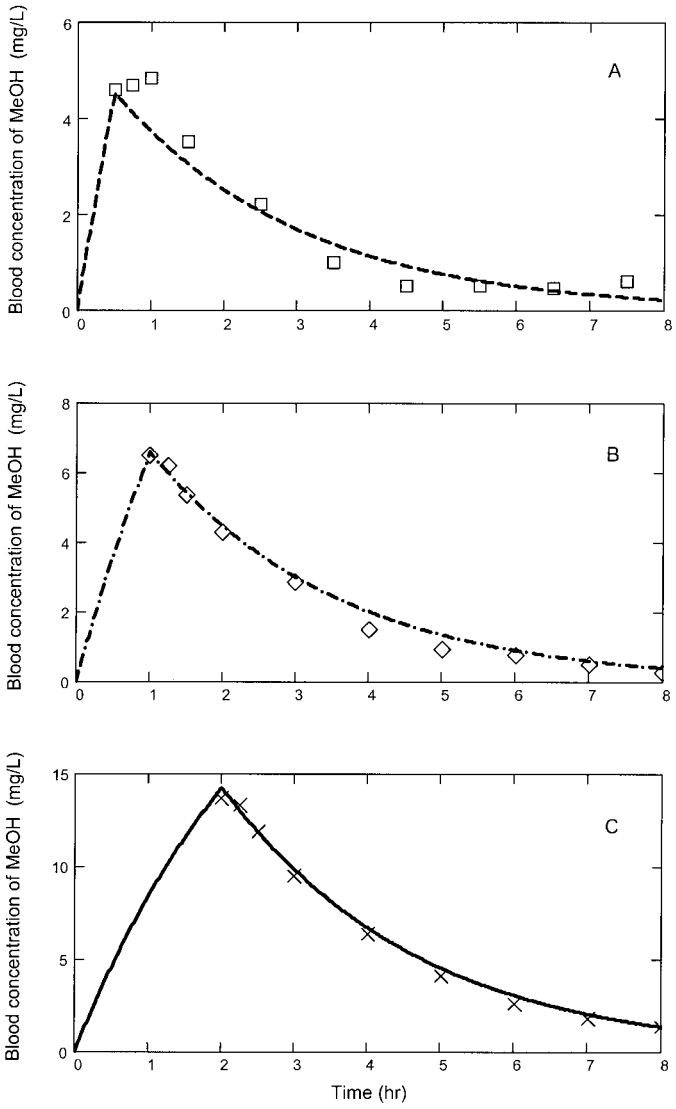

FIG. 9. Comparison of model simulations (lines) with experimental data (symbols represent mean values from 4 subjects) of Batterman *et al.* (1998) on the blood concentration-time profiles of methanol in 3 groups of human volunteers during and following inhalation exposures to 800 ppm of methanol vapors for 30 min (A), 1 h (B), and 2 h (C).

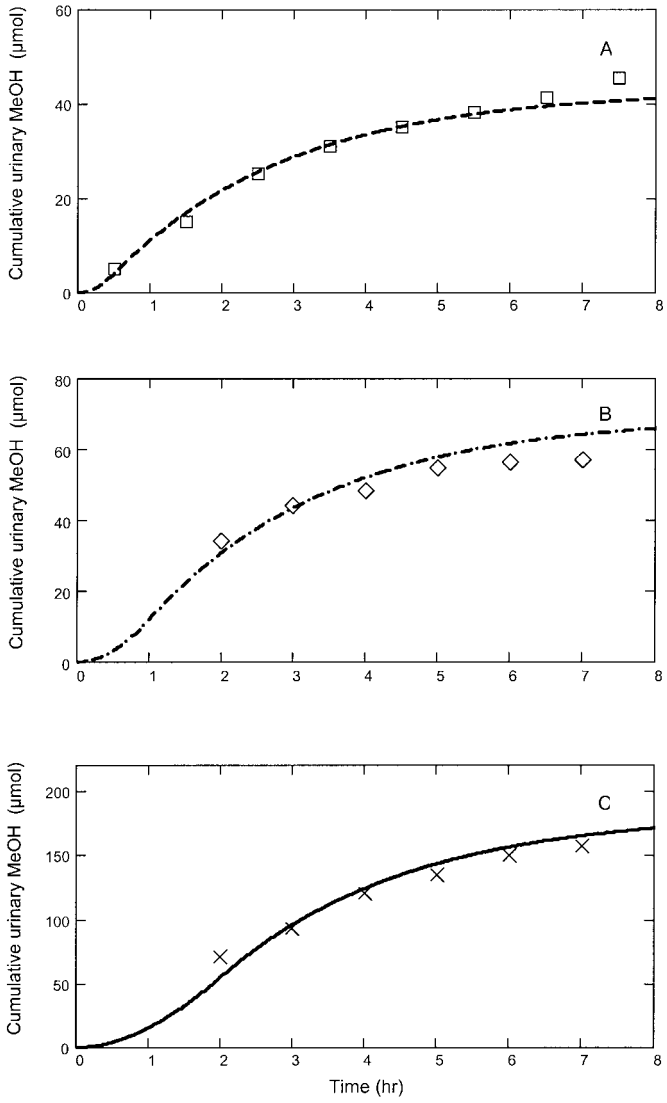

FIG. 10. Comparison of model simulations (lines) with experimental data (symbols represent mean values from 4 subjects) of Batterman *et al.* (1998) on the cumulative urinary excretion time courses of methanol in 3 groups of human volunteers during and following inhalation exposures to 800 ppm of methanol vapors for 30 min (A), 1 h (B), and 2 h (C).

blood and urine during a continuous inhalation exposure to 200 ppm of methanol, considering a negligible background burden of methanol, an absorption fraction of 0.577, a pulmonary ventilation rate of 10.8 l/min, and a daily urinary excretion rate of 1.5 l. Near steady state levels were reached within 20 h following the start of exposure. At the end of a 5-day exposure period, predicted blood concentration of methanol was 5.5 mg/l (171 μmol/l) and that of formate was 0.16 mg/l (3.5 μmol/l). The latter formate concentration was obtained by considering an apparent volume of distribution of formate in humans (in l/kg of body weight) similar to that calculated for monkeys. With this exposure level, the model predicts near steady state urinary concentrations of methanol of 8.1 mg/l (252 μmol/l) and of formic acid of 1.5 mg/l (31.7 μmol/l, 0.97 mg/g creatinine, or 2390 μmol/mol creatinine).

Thus, at the end of a 5-day continuous inhalation exposure to 200 ppm of methanol vapors, predicted methanol concentrations in blood and urine were 5 to 11 times greater than reported mean background values of unexposed subjects (1 mg/l in blood and 0.73 mg/l in urine) (Osterloh *et al.*, 1996; Sedivec *et al.*, 1981). On the other hand, predicted concentrations of blood formate and urinary formic acid in humans (0.16 and 1.5 mg/l, respectively), although in accordance with the experimental data from methanol exposures in primates and humans, were well below mean background values of unexposed subjects (4.9–10.3 mg/l in blood and 6.3–13 mg/l in

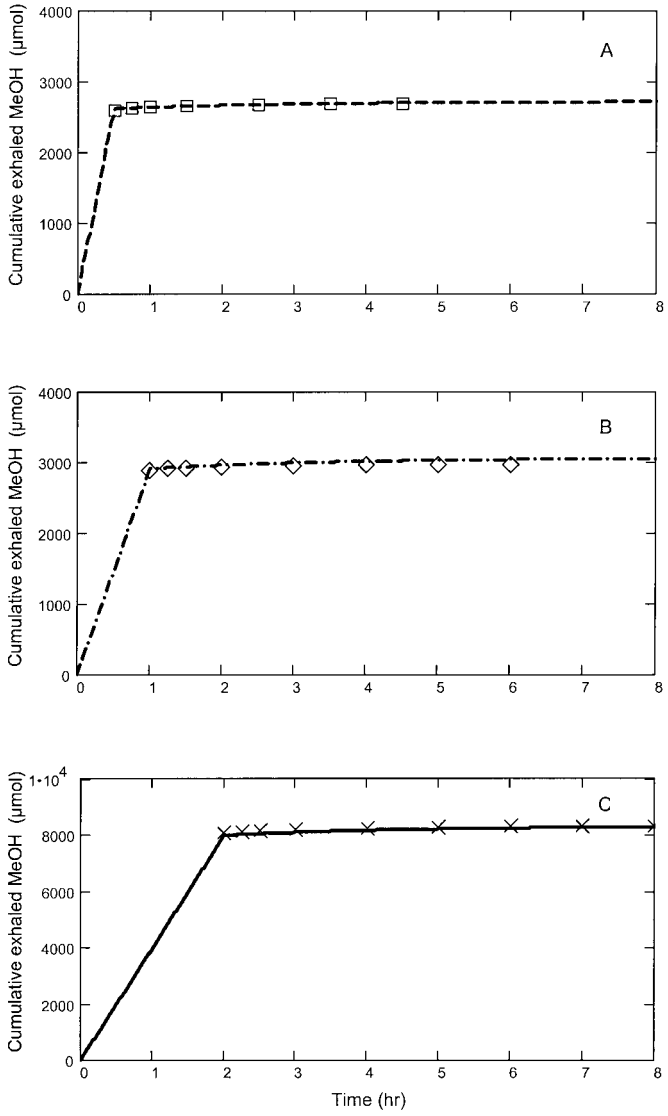

FIG. 11. Comparison of model simulations (lines) with experimental data (symbols represent mean values from 4 subjects) of Batterman *et al.* (1998) on the cumulative exhalation time courses of methanol in 3 groups of human volunteers during and following inhalation exposures to 800 ppm of methanol vapors for 30 min (A), 1 h (B), and 2 h (C).

urine) reported by various authors (Baumann and Angerer, 1979; D'Alessandro *et al.,* 1994; Heinrich and Angerer, 1982; Lee *et al.,* 1992; Osterloh *et al.,* 1996).

The model simulations suggest that an 8-h inhalation exposure of at least 500 to 2000 ppm, without physical activities, would be necessary for blood formate and urinary formic acid concentrations to reach reported mean background values. The exact exposure levels necessary depend on the values assumed for the absorption fraction, the pulmonary ventilation rate, and the daily urinary excretion rate. There are considerable variations in the literature for these parameters.

DISCUSSION

Model Description of the Kinetics of Methanol and Its Metabolites

A biologically based dynamic model was developed to simulate the uptake and disposition of methanol and its metabolites (formaldehyde, formate, CO_2) in animals and humans. Based on the *in vivo* time profiles of methanol, formate, and CO_2 in blood and accessible biological matrices, the model was able to reproduce the essential kinetic processes of methanol disposition. It can now be used to quantitatively relate the parent compound or the metabolites in biological matrices to the absorbed dose and tissue burdens at any point in time in rats, monkeys, and humans for different exposure situations, thus reducing the uncertainties in the dose-response relationship, animal-to-human, and exposure scenario comparisons.

The model showed that the kinetics of systemic methanol were dependent on the pulmonary uptake and on the metabolism of methanol to formaldehyde. The pulmonary absorption fraction and ventilation rate were the only model parameters that needed to be modified within a species to provide a good prediction of all the data sets. These predicted parameters were, however, in the value range reported in the published literature (Dorman *et al.,* 1994; Fisher *et al.,* 2000; Horton *et al.,* 1992; Sedivec *et al.,* 1981). The pulmonary absorption fraction did not appear to be influenced by the exposure level or duration nor by the pulmonary ventilation rate, as observed previously by Sedivec *et al.* (1981) and Medinsky *et al.* (1997).

For all the other parameters, a single set of values for a given species and strain was found to provide a close approximation to the available kinetic time profile data. In particular, a single average value for the metabolism rate constants can be used in the model for a given exposure route. In accordance with the published animal data (Dorman *et al.,* 1994; Horton *et al.,* 1992), the model predicts that absorbed methanol is eliminated mainly by metabolism to formaldehyde and that only a minor fraction of the exposure dose is eliminated as unchanged methanol in urine.

On the other hand, from the rat data of Horton *et al.* (1992), differences in the saturation of methanol metabolism were observed depending on the exposure route. Indeed, in the rat model, a same value for the metabolism rate constant of whole body methanol to formaldehyde, k_{met}, was found to provide a close approximation to the data of Horton *et al.* (1992) on the blood concentration-time profiles of methanol after iv and inhalation exposures to methanol in Fischer-344 rats. However, the K_m value determined using the inhalation data was 3 times smaller than that obtained using the iv data. Although methanol has been reported to be metabolized mainly in the liver, pulmonary metabolism is also likely to occur. Indeed, the catalase-peroxidase system responsible for a major fraction of methanol metabolism in rats is widely distributed in mammalian tissues (Housset, 1986; Morikawa and Harada, 1969; Sugata *et al.,* 1979). It is, in particular, present in the membranes of the upper respiratory tract, the main site of pulmonary absorption of methanol (Perkins *et al.,* 1996). Of course, given that the K_m parameters were estimated from mean blood concentration data without taking into account interindividual variations, it cannot be excluded that there is no significant difference between the 2 route-specific K_m values.

It should be remembered that, only in the case of methanol metabolism to formaldehyde was a saturation constant necessary. As mentioned previously, for the exposure dose range of the studies on which the model is based, no saturation of formate or CO_2 metabolism was apparent (Dorman *et al.,* 1994; Horton *et al.,* 1992; Osterloh *et al.,* 1996; Sedivec *et al.,* 1981). Though the saturation of formate metabolism has been reported after very high iv doses of sodium formate in rats (164, 328, and 492 mg/kg; Damian and Raabe, 1996) and appeared to occur in a case of methanol poisoning in a human subject (Jacobsen *et al.,* 1988), under inhalation exposures levels in occupational and general environments, it is unlikely to occur.

According to model predictions, congruent with the data in the literature (Dorman *et al.,* 1994; Horton *et al.,* 1992), a certain fraction of formaldehyde is readily oxidized to formate, a major fraction of which is rapidly converted to CO_2 and exhaled, whereas a small fraction is excreted as formic acid in urine. However, fits to the available data in rats and monkeys of Horton *et al.* (1992) and Dorman *et al.* (1994) show that, once formed, a substantial fraction of formaldehyde is converted to unobserved forms. This pathway contributes to a long-term unobserved compartment. The latter, most plausibly, represents either the formaldehyde that (directly or after oxidation to formate) binds to various endogenous molecules (Heck *et al.,* 1983; Røe, 1982) or is incorporated in the tetrahydrofolic-acid-dependent one-carbon pathway to become the building block of a number of synthetic pathways (Røe, 1982; Tephly and McMartin, 1984). That substantial amounts of methanol metabolites or by-products are retained for a long time is verified by Horton *et al.* (1992) who estimated that 18 h following an iv injection of 100 mg/kg of ^{14}C-methanol in male Fischer-344 rats, only 57% of the dose was eliminated from the body. From the data of Dorman *et al.* (1994) and Medinsky *et al.* (1997), it can further be calculated that 48 h following the

start of a 2-h inhalation exposure to 900 ppm of ^{14}C-methanol vapors in female cynomolgus monkeys, only 23% of the absorbed ^{14}C-methanol was eliminated from the body. These findings are corroborated by the data of Heck *et al.* (1983) showing that 40% of a ^{14}C-formaldehyde inhalation dose remained in the body 70 h postexposure.

In the present study, the model proposed rests on acute exposure data, where the time profiles of methanol and its metabolites were determined only over short time periods (a maximum of 6 h of exposure and a maximum of 48 h postexposure). This does not allow observation of the slow release from the long-term components.

It is to be noted that most of the published studies on the detailed disposition kinetics of methanol regard controlled short-term (iv injection or continuous inhalation exposure over a few hours) methanol exposures in rats, primates, and humans (Batterman *et al.,* 1998; Damian and Raabe, 1996; Dorman *et al.,* 1994; Ferry *et al.,* 1980; Fisher *et al.,* 2000; Franzblau *et al.,* 1995; Horton *et al.,* 1992; Jacobsen *et al.,* 1988; Osterloh *et al.,* 1996; Pollack *et al.,* 1993; Sedivec *et al.,* 1981; Ward *et al.,* 1995; Ward and Pollack, 1996). Experimental studies on the detailed time profiles following controlled repeated exposures to methanol are lacking. Data on methanol and formate concentrations in spot blood and urine samples of chronically exposed workers (Baumann and Angerer, 1979; Kawai *et al.,* 1991; Yasugi *et al.,* 1992) are available but uncertainties regarding the exposure dose and concomitant exposure to other chemicals limit their use in the elaboration of a kinetic model.

With regard to the apparent volume of distribution of methanol, which was calculated in the current study using classic approaches (see Method and Model Presentation), it was expected that its value would correspond approximately to the whole body water content. The slightly larger weight adjusted volume of distribution of methanol calculated in rats (0.92 l/kg of body weight) as compared to monkeys (0.77 l/kg of body weight) can be explained by the smaller adipose tissue fraction of body weight in rats.

As for the apparent volume of distribution of formate determined in this study, the weight adjusted values calculated in rats and monkeys were in the same range, although slightly higher in rats than in monkeys (6.4 and 4.6 l/kg of body weight, respectively). However, the volume of distribution of formate was larger than that of methanol, which strongly suggests that formate distributes in body constituents other than water, such as proteins. The closeness of our simulations to the available experimental data on the time course of formate blood concentrations is consistent with the volume of distribution concept (i.e., rapid exchanges between the nonblood pool of formate and blood formate).

Species Differences in the Kinetics of Methanol and Its Metabolites

Critical biological determinants of species differences in the disposition of methanol and its metabolites were determined from *in vivo* data from several studies (Batterman *et al.,* 1998; Fisher *et al.,* 2000; Horton *et al.,* 1992; Sedivec *et al.,* 1981). In agreement with their findings, the model predicts that the average pulmonary absorption fraction f_{abs} of methanol and the metabolism rate constant k_{met} of whole body methanol to formaldehyde were in the same value range in rats, monkeys, and humans (on average 0.58–0.82 for f_{abs} and 0.219–0.96/h for k_{met}). However, the saturation of methanol metabolism appeared to occur at a lower exposure dose in rats than in monkeys and humans. Indeed, from the data of Horton *et al.* (1992) on the blood concentration-time profile of methanol in rats exposed to 2000 ppm of methanol vapors for 6 h, a K_m value of 36.6 mg/l of blood and V_{max} of 19.4 mg/l/h were estimated in the current study whereas following a similar exposure in monkeys, no saturation of methanol metabolism was apparent. The model also predicts that there is no saturation of methanol metabolism from the data of Batterman *et al.* (1998) in human volunteers exposed to 800 ppm of methanol vapors for 2 h nor from those of Sedivec *et al.* (1981) in volunteers exposed to 229 ppm of methanol vapors for 8 h.

Interestingly, a striking species difference in the kinetics was attributed to a metabolism rate constant ratio k_{form}/k_{fald} of whole body formaldehyde to formate twice as high in rats than in monkeys (0.53 vs. 0.26). Thus, in monkeys and plausibly humans, a much larger fraction of body formaldehyde is rapidly converted to unobserved forms rather than passed on to formate and eventually CO_2.

Comparison of the Current Model with Others Previously Published

The current biologically based dynamic model can be compared to some of the previously published models. In particular, Horton *et al.* (1992) developed a PBPK model to describe the kinetics of methanol and its metabolites in rats, monkeys, and humans. Their model was comprised of 4 compartments: liver, kidney, and richly and slowly perfused tissues. As in our model, the metabolism of methanol to formaldehyde was assumed to be the main biological determinant of methanol elimination kinetics. In addition, in both the current model and that published by Horton *et al.* (1992), not only were the kinetics of methanol in blood, urine, and exhaled air modeled but also the time evolution of formate in blood and urine and of CO_2 in the exhaled air. However, in the study of Horton *et al.* (1992), 2 saturable metabolic pathways for methanol metabolism were considered whereas in our study, even by introducing only 1 metabolism route for methanol, with saturable elimination, the model gave a good prediction of the experimental data. In the PBPK model of Horton *et al.* (1992), the metabolism of formate and CO_2 was also assumed to follow Michaelis Menten kinetics. In our model, as mentioned previously, no saturation constants for these metabolism processes were introduced since fits to the available time course data suggested the absence of saturation of formate and CO_2 me-

tabolism in the exposure dose range used in the studies on which the model is based.

Furthermore, conceptual and functional differences between the current model and the PBPK model of Horton *et al.* (1992) are related to the fact that the current model compartmentalization is dependent on the availability of experimental data on the detailed time course of methanol and its metabolites in blood, tissues, and excreta and on the hierarchy of the time scales for the various biological processes. The main structural difference between our model and that of Horton *et al.* (1992) concerns our regrouping into a single compartment the methanol body burden whereas Horton *et al.* (1992) have fragmented the body into several compartments according to the general PBPK structure. In our model, methanol body burden regrouping relies on the fact that methanol distributes uniformly and rapidly in total body water and thus the apparent volume of distribution of methanol corresponds to the total body water content. This allowed us to reduce the number of parameters to be determined to describe the overall model dynamics of methanol. Based on the available data on methanol blood kinetics for the 3 species studied, this regrouping also enabled the determination of species specific parameters by direct fits, without the need for allometric extrapolation. Furthermore, the current model ensures conservation of mass by the introduction of an unobserved metabolite compartment. In the model of Horton *et al.* (1992), to account for the fraction of the methanol dose that was unobserved experimentally and thus to obtain a good fit to their experimental data on the cumulative exhalation of CO_2 in rats exposed to ^{14}C-labeled methanol, the rate of formate metabolism had to be multiplied by 0.6 to correspond to the fraction of the methanol dose eventually excreted as CO_2 over the 18-h sample collection period of their study.

More recently, Fisher *et al.* (2000) published a PBPK model for monkeys to describe the kinetics of methanol. The structure of their PBPK model for methanol was similar to that of Horton *et al.* (1992) but also accounted for the fractional systemic uptake of inhaled methanol vapors in the lungs. This fractional systemic uptake was also introduced in our model.

Prediction of the Most Useful Biological Indicator of Exposure to Methanol

The biological monitoring of exposure, through the analysis of blood concentrations or urinary and exhaled levels, has become an increasingly popular means of estimating the absorbed dose. This model can be used in conjunction with biological measurements of methanol and formate or formic acid to determine the level of exposure and subsequent build-up in tissues. It can also help to establish the best biomarker of exposure, the sampling strategy for routine monitoring and the significance of measurements at different times.

Since systemic formate is thought to be responsible for a large part of the deleterious effects induced by methanol exposures (Tephly and McMartin, 1984), the measurement of blood formate or urinary formic acid appears interesting *a priori* for the biological monitoring of exposure to methanol. However, the model shows that background concentrations of formate are much higher than those stemming from fairly high methanol exposures. Indeed, the model, adapted to kinetic data in human volunteers exposed acutely to methanol vapors, predicts that 8-h inhalation exposures ranging from 500 to 2000 ppm are needed to increase blood formate concentrations above reported mean endogenous values of 4.9 to 10.3 mg/l (Baumann and Angerer, 1979; Lee *et al.*, 1992; Osterloh *et al.*, 1996), and for urinary formic acid concentrations to reach the published mean background values of 6.3 to 13 mg/l (Baumann and Angerer, 1979; D'Alessandro *et al.*, 1994; Heinrich and Angerer, 1982). The monkey data of Dorman *et al.* (1994) show that even after a 2-h inhalation exposure to 900 ppm of ^{14}C-methanol in female cynomolgus monkeys, ^{14}C-formate concentrations in blood were far below normal endogenous values. Likewise, studies in human volunteers acutely exposed to methanol, at the level of 200 ppm, concur to indicate that blood formate and urinary formic acid concentrations remain within the background value range of unexposed subjects (D'Alessandro *et al.*, 1994; Franzblau *et al.*, 1993; Lee *et al.*, 1992; Osterloh *et al.*, 1996).

Only in the studies of Kawai *et al.* (1991) and Yasugi *et al.* (1992) was a significant correlation between the urinary excretion of formic acid and exposure to methanol vapors observed. However, the workers were exposed to airborne concentrations of methanol of up to 4000 ppm over an 8-h workshift.

These findings suggest that it is not justified to monitor concentrations of blood formate or urinary formic acid at methanol exposure levels in the range of or below the airborne threshold limit value of 200 ppm for occupational settings. If toxic effects do occur following low level methanol exposures, the mode of action is not likely to be through the accumulation of formate. As suggested by some reports (Cook *et al.*, 1991; Kingsley and Hirsch, 1954), it may rather be attributable to methanol itself.

The use of formate as a biomarker of exposure to methanol is further limited by the fact that it is not a specific metabolite of methanol exposure. Also, background concentrations of formate are subject to wide interindividual variations (Baumann and Angerer, 1979; D'Alessandro *et al.*, 1994; Franzblau *et al.*, 1995; Heinrich and Angerer, 1982; Lee *et al.*, 1992; Osterloh *et al.*, 1996; Sedivec *et al.*, 1981). This leaves blood and urinary methanol as the most appropriate biomarkers of absorbed methanol. Since the model relates blood and urinary methanol burdens to the exposure dose and body burdens of metabolites at all time points, it can be of great use in reconstructing past and present exposure levels starting from methanol amounts in blood and urine.

APPENDIX

Methanol Kinetics: First Order Linear Differential Equations for Each Compartment

Kinetics of the Methanol Form

From Figure 1, the following differential equations are obtained (see Table 1 for definitions of symbols):

$$\frac{dL(t)}{dt} = -(k_{abs} + k_{re}) \times L(t) + g(t) \quad (1)$$

where g(t) is the pulmonary exposure dose per unit of time. $g(t) = C_{exp} \times VR$ where C_{exp} is the exposure concentration and VR is the pulmonary ventilation rate. For an iv injection, $g(t) = 0$ for $t > 0$ and at time $t = 0$, $X(0) = 100\%$ of dose.

The fraction of absorption through the lungs can be defined as $f_{abs} = k_{abs}/(k_{abs} + k_{re})$.

$$\frac{dX(t)}{dt} = k_{abs} \times L(t) - (k_m + k_{ex}) \times X(t) - k_{met} \times h(X) \times X(t) \quad (2)$$

where

$$h(X) = \frac{K_m}{K_m + X(t)}$$

Considering the rapid exchange rates between the various internal organs and blood, and thus between the whole body burden and blood, it can be considered that

$$X(t) = V_d^{MeOH} \times B(t) \quad (3)$$

where B(t) is the blood concentration of methanol as a function of time.

$$\frac{dE(t)}{dt} = k_{re} \times L(t) + k_{ex} \times X(t) \quad (4)$$

$$\frac{dM(t)}{dt} = k_m \times X(t) \quad (5)$$

Kinetics of the Formaldehyde Form

$$\frac{dY(t)}{dt} = k_{met} \times h(X) \times X(t) - (k_{form} + k_{oth}) \times Y(t) \quad (6)$$

The global breakdown rate of formaldehyde, $k_{fald} = k_{form} + k_{oth}$, is very large compared to the subsequent transfer rates $\{k_u$ and $k_{CO2}\}$ because observed formaldehyde levels are very small compared to formate levels. This implies rapid breakdown of formaldehyde compared to formate elimination rates. The rate of formaldehyde breakdown, k_{fald}, was given the value reported by McMartin *et al.* (1979) in cynomolgus monkeys, corresponding to a half-life of 1.5 min. However, its exact value is not relevant to the model's unfolding, only the ratios k_{form}/k_{fald} and k_{oth}/k_{fald} are.

Kinetics of the Other Forms

$$\frac{dO(t)}{dt} = k_{oth} \times Y(t) - k_n \times O(t) \quad (7)$$

$$\frac{dN(t)}{dt} = k_n \times O(t) \quad (8)$$

$$\frac{dU(t)}{dt} = \frac{dO(t)}{dt} + \frac{dN(t)}{dt} = k_{oth} \times Y(t) \quad (9)$$

Kinetics of the Formate Form

$$\frac{dZ(t)}{dt} = k_{form} \times Y(t) - (k_u + k_{CO2}) \times Z(t) \quad (10)$$

$$\frac{dF(t)}{dt} = k_u \times Z(t) \quad (11)$$

$$\frac{dC(t)}{dt} = k_{CO2} \times Z(t) \quad (12)$$

Mass Balance Verification

$$E(t) + L(t) + X(t) + M(t) + Y(t) + U(t) + Z(t) + F(t) + C(t) - (E(0) + L(0) + X(0) + M(0) + Y(0) + U(0) + Z(0) + F(0) + C(0)) = \int_0^t g(t) \times dt = \text{total exposure dose over time}$$

REFERENCES

Batterman, S. A., Franzblau, A., D'Arcy, J. B., Sargent, N. E., Gross, K. B., and Schreck, R. M. (1998). Breath, urine, and blood measurements as biological exposure indices of short-term inhalation exposure to methanol. *Int. Arch. Occup. Environ. Health* **71,** 325–335.

Baumann, K., and Angerer, J. (1979). Occupational chronic exposure to organic solvents. VI. Formic acid concentration in blood and urine as an indicator of methanol exposure. *Int. Arch. Occup. Environ. Health* **42,** 241–249.

Bolon, B., Dorman, D. C., Janszen, D., Morgan, K. T., and Welsch, F. (1993). Phase-specific developmental toxicity in mice following maternal methanol inhalation. *Fundam. Appl. Toxicol.* **21,** 508–516.

Carrier, G., Bouchard, M., Brunet, R. C., and Caza, M. (2001a). A toxicokinetic model for predicting the tissue distribution and elimination of organic and inorganic mercury following exposure to methyl mercury in animals and humans. II. Application and validation of the model in humans. *Toxicol. Appl. Pharmacol.* **171,** 50–60.

Carrier, G., and Brunet, R. C. (1999). A toxicokinetic model to assess the risk of azinphosmethyl exposure in humans through measures of urinary elimination of alkylphosphates. *Toxicol. Sci.* **47,** 23–32.

Carrier, G., Brunet, R. C., and Brodeur, J. (1995a). Modeling of the toxicokinetics of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in mammalians, including humans. I. Nonlinear distribution of PCDD/PCDF body

burden between liver and adipose tissues. *Toxicol. Appl. Pharmacol.* **131,** 253–266.

Carrier, G., Brunet, R. C., and Brodeur, J. (1995b). Modeling of the toxicokinetics of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in mammalians, including humans. II. Kinetics of absorption and disposition of PCDDs/PCDFs. *Toxicol. Appl. Pharmacol.* **131,** 267–276.

Carrier, G., Brunet, R. C., Caza, M., and Bouchard, M. (2001b). A toxicokinetic model for predicting the tissue distribution and elimination of organic and inorganic mercury following exposure to methyl mercury in animals and humans. I. Development and validation of the model using experimental data in rats. *Toxicol. Appl. Pharmacol.* **171,** 38–49.

Cook, M. R., Bergman, F. J., Cohen, H. D., Gerkovich, M. M., Graham, C., Harris, R. K., and Siemann, L. G. (1991). *Effects of Methanol Vapor on Human Neurobehavioral Measures.* Research Report Number 42. Health Effects Institute, Cambridge, MA.

D'Alessandro, A., Osterloh, J. D., Chuwers, P., Quinlan, P. J., Kelly, T. J., and Becker, C. E. (1994). Formate in serum and urine after controlled methanol exposure at the threshold limit value. *Environ. Health Perspect.* **102,** 178–181.

Damian, P., and Raabe, O. G. (1996). Toxicokinetic modeling of dose-dependent formate elimination in rats: *In vivo-in vitro* correlations using the perfused rat liver. *Toxicol. Appl. Pharmacol.* **139,** 22–32.

Dorman, D. C., Moss, O. R., Farris, G. M., Janszen, D., Bond, J. A., and Medinsky, M. A. (1994). Pharmacokinetics of inhaled [^{14}C]methanol and methanol-derived [^{14}C]formate in normal and folate-deficient cynomolgus monkeys. *Toxicol. Appl. Pharmacol.* **128,** 229–238.

Downie, A., Khattab, T. M., Malik, M. I. A., and Samara, I. N. (1992). A case of percutaneous industrial methanol toxicity. *Occup. Med.* **42,** 47–49.

Ferry, D. G., Temple, W. A., and McQueen, E. G. (1980). Methanol monitoring. Comparison of urinary methanol concentration with formic acid excretion rate as a measure of occupational exposure. *Int. Arch. Occup. Environ. Health* **47,** 155–163.

Fisher, J. W., Dorman, D. C., Medinsky, M. A., Welsch, F., and Conolly, R. B. (2000). Analysis of respiratory exchange of methanol in the lung of the monkey using a physiological model. *Toxicol. Sci.* **53,** 185–193.

Franzblau, A., Batterman, S. A., D'Arcy, J. B., Sargent, N. E., Gross, K. B., and Schreck, R. M. (1995). Breath monitoring of inhalation and dermal methanol exposure. *Appl. Occup. Environ. Hyg.* **10,** 833–839.

Franzblau, A., Lee, E. W., Schreck, R. M., D'Arcy, J. B., Santrock, J., and Levine, S. P. (1993). Absence of formic acid accumulation in urine following five days of methanol exposure. *Appl. Occup. Environ. Hyg.* **8,** 883–888.

Goodman, J. I., and Tephly, T. R. (1968). The role of hepatic microbody and soluble oxidases in the peroxidation of methanol in the rat and monkey. *Mol. Pharmacol.* **4,** 492–501.

Health Effects Institute (1987). *Automotive Methanol Vapors and Human Health: An Evaluation of Existing Scientific Information and Issues for Future Research (a Special Report)*. Health Effects Institute, Cambridge, MA.

Heck, H. A., Chin, T. Y., and Schmitz, M. C. (1983). Distribution of [^{14}C] formaldehyde in rats after inhalation exposure. In *Formaldehyde Toxicity* (J. E. Gibson, Ed.), pp. 26–37. Hemisphere Publishing Corporation, Washington, DC.

Heinrich, R., and Angerer, J. (1982). Occupational chronic exposure to organic solvents. X. Biological monitoring parameters for methanol exposure. *Int. Arch. Occup. Environ. Health* **50,** 341–349.

Horton, V. L., Higuchi, M. A., and Rickert, D. E. (1992). Physiologically based pharmacokinetic model for methanol in rats, monkeys, and humans. *Toxicol. Appl. Pharmacol.* **117,** 26–36.

Housset, B. (1986). Mechanisms of oxygen-induced lung injury. *Arch. Int. Physiol. Biochim.* **94,** S1–5.

IPCS (1997). *Environmental Health Criteria 196–Methanol*. International Programme on Chemical Safety, World Health Organization, Geneva.

Jacobsen, D., Webb, R., Collins, T. D., and McMartin K. E. (1988). Methanol and formate kinetics in late diagnosed methanol intoxication. *Med. Toxicol. Adverse Drug Exp.* **3,** 418–423.

Kavet, S., and Nauss, K. M. (1990). The toxicity of inhaled methanol vapors. *Crit. Rev. Toxicol.* **21,** 21–50.

Kawai, T., Yasugi, T., Mizunuma, K., Horiguchi, S., Hirase, Y., Uchida, Y., and Ikeda, M. (1991). Methanol in urine as a biological indicator of occupational exposure to methanol vapor. *Int. Arch. Occup. Environ. Health* **63,** 311–318.

Kingsley, W. H., and Hirsch, F. G. (1954). Toxicologic considerations in direct process spirit duplicating machines. *Comp. Med.* **6,** 7–8.

Knuiman, J. T., Hautvast, A. J., van der Heyden, L., Geboers, J., Joossens, J. V., Tornquist, H., Isaksson, B., Pietinen, P., Tuomilehto, U., Poulsen, L., Flynn, A., Shortt, C., Böing, H., Yomtov, B., Magalhaes, E., Angelico, F., Stefanutti, C., Fazio, C., Cantini, R., Ricci, G., Trichopoulou, A., and Katapoti, J. (1986). A multi-centre study on completeness of urine collection in 11 European centres. I. Some problems with the use of creatinine and 4-aminobenzoic acid as marker of the completeness of collection. *Human Nutr. Clin. Nutr.* **40,** 229–237.

Lee, E. W., Terzo, T. S., D'Arcy, J. B., Gross, K. B., and Schreck, R. M. (1992). Lack of blood formate accumulation in humans following exposure to methanol vapor at the current permissible exposure limit of 200 ppm. *Am. Ind. Hyg. Assoc. J.* **53,** 99–104.

Liesivuori, J., and Savolainen, H. (1991). Methanol and formic acid toxicity: Biochemical mechanisms. *Pharmacol. Toxicol.* **69,** 157–163.

McMartin, K. E., Martin-Amat, G., Makar, A. B., and Tephly, T. R. (1977). Methanol poisoning. V. Role of formate metabolism in the monkey. *J. Pharmacol. Exp. Ther.* **201,** 564–572.

McMartin, K. E., Martin-Amat, G., Noker, P. E., and Tephly, T. R. (1979). Lack of role for formaldehyde in methanol poisoning in the monkey. *Biochem. Pharmacol.* **28,** 645–649.

Medinsky, M. A., Dorman, D. C., Bond, J. A., Moss, O. R., Janszen, D. B., and Everitt, J. I. (1997). Pharmacokinetics of methanol and formate in female cynomolgus monkeys exposed to methanol vapors. *Res. Rep. Health Eff. Inst.* **77,** 1–30; discussion 31–38.

Morikawa, S., and Harada, T. (1969). Immunohistochemical localization of catalase in mammalian tissues. *J. Histochem. Cytochem.* **17,** 30–5.

Nelson, B. K., Brightwell, S., MacKenzie, D. R., Khan, A., Burg, J. R., Weigel, W. W., and Goad, P. T. (1985). Teratological assessment of methanol and ethanol at high inhalation levels in rats. *Fundam. Appl. Toxicol.* **5,** 727–736.

Nihlén, A., and Droz, P.-O. (2000). Toxicokinetic modelling of methyl formate exposure and implications for biological monitoring. *Int. Arch. Occup. Environ. Health* **73,** 479–487.

Osterloh, J. D., D'Alessandro, A., Chuwers, P., Mogadeddi, H., and Kelly, T. J. (1996). Serum concentrations of methanol after inhalation at 200 ppm. *J. Occup. Environ. Med.* **38,** 571–576.

Palese, M., and Tephly, T. R. (1975). Metabolism of formate in the rat. *J. Toxicol. Environ. Health* **1,** 13–24.

Perkins, R. A., Ward, K. W., and Pollack, G. M. (1995). A pharmacokinetic model of inhaled methanol in humans and comparison to methanol disposition in mice and rats. *Environ. Health Perspect.* **103,** 726–733.

Perkins, R. A., Ward, K. W., and Pollack, G. M. (1996). Methanol inhalation: Site and other factors influencing absorption, and an inhalation toxicokinetic model for the rat. *Pharm. Res.* **13,** 749–55.

Pollack, G. M., and Brouwer, K. L. (1996). Maternal-fetal pharmacokinetics of methanol. *Res. Rep. Health Eff. Inst.* **74,** 1–48; discussion 49–53.

Pollack, G. M., Brouwer, K. L., and Kawagoe, J. L. (1993). Toxicokinetics of

intravenous methanol in the female rat. *Fundam. Appl. Toxicol.* **21,** 105–110.

Røe, O. (1982). Species differences in methanol poisonings. *Crit. Rev. Toxicol.* **10,** 275–286.

Sedivec, V., Mraz, M., and Flek, J. (1981). Biological monitoring of persons exposed to methanol vapours. *Int. Arch. Occup. Environ. Health* **48,** 257–271.

Sejersted, O. M., Jacobsen, D., Ovrebo, S., and Jansen, H. (1983). Formate concentrations in plasma from patients poisoned with methanol. *Acta Med. Scand.* **213,** 105–110.

Sugata, Y., Halbach, S., Allen, J., and Clarkson, T. W. (1979). Tissue distribution of inhaled mercury vapor in acatalasemic mice. *J. Environ. Sci. Health C* **13,** 97–106.

Tephly, T. R., and McMartin, K. E. (1984). Methanol metabolism and toxicity. In *Aspartame. Physiology and Biochemistry* (L. D. Stegink and L. J. Filer, Jr., Eds.), pp. 111–140. Marcel Dekker, New York.

U.S. DHHS (1993). Methanol toxicity. Agency for Toxic Substances and Disease Registry. *Am. Fam. Physician* **47,** 163–171.

Ward, K. W., Blumenthal, G. M., Welsch, F., and Pollack, G. M. (1997). Development of a physiologically based pharmacokinetic model to describe the disposition of methanol in pregnant rats and mice. *Toxicol. Appl. Pharmacol.* **145,** 311–322.

Ward, K. W., Perkins, R. A., Kawagoe, J. L., and Pollack, G. M. (1995). Comparative toxicokinetics of methanol in the female mouse and rat. *Fundam. Appl. Toxicol.* **26,** 258–264.

Ward, K. W., and Pollack, G. M. (1996). Comparative toxicokinetics of methanol in pregnant and nonpregnant rodents. *Drug. Metab. Dispos.* **24,** 1062–1070.

Yasugi, T., Kawai, T., Mizunuma, K., Horiguchi, S., Iwami, O., Iguchi, H., and Ikeda, M. (1992). Formic acid excretion in comparison with methanol excretion in urine of workers occupationally exposed to methanol. *Int. Arch. Occup. Environ. Health* **64,** 329–37.