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Development and Use of PBPK Modeling and the Impact of Metabolism on Variability in Dose Metrics for the Risk Assessment of Methyl Tertiary Butyl Ether (MTBE)

Jerry N. Blancato[1*], Marina V. Evans[2], Fred W. Power[3], and Jane C. Caldwell[4]

[1]*National Center for Computational Toxicology, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711*; [2]*National Health and Environmental Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711*; [3]*National Exposure Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Las Vegas Nevada*; [4]*National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC 20460*

Abstract

MTBE is a volatile organic compound (VOC) that is used as an oxygenate additive to gasoline. This chemical has been detected in groundwater and in surface water reservoirs due to leaks and contamination. MTBE is rapidly absorbed through both oral and inhalation exposure routes, is rapidly distributed throughout the body, and metabolized by two different enzyme pathways in the liver. Kinetic studies have focused mainly on the inhalation pathway and tracked concentrations of parent compound and the MTBE metabolite, tertiary butyl alcohol (TBA). PBPK models are used to predict the toxicologically relevant dose and to extrapolate across different routes and species. In this paper, we report improvements to existing rodent and human PBPK models, and compare model predictions with available experimental data. The criteria for the selection of the human database and analysis making use of Haber's rule are described. Using the rodent model, a sensitivity analysis revealed metabolism to be one of 22 parameters affecting MTBE and TBA blood concentrations at relatively high concentrations. A Monte Carlo analysis that used the same exposure conditions and varied the same parameters in the human model suggested that TBA blood concentrations were more affected than those of MTBE. An impact analysis (impact is defined as an examination of changes in dose metric predictions resulting from the alteration of one or more parameters) using the human model but at exposure levels consistent with environmental and human experimental study levels was performed to study the possible effects of varying only metabolic parameters. Inhalation exposures were examined, and the dose metrics were: peak MTBE in venous blood, AUC in venous blood at 24 hours, amount of MTBE metabolized in the liver at 24 hours, and peak TBA concentration in venous blood. Extrapolated rat metabolic parameters or scaled human *in vitro* metabolic values were compared for predictions of peak MTBE and TBA blood concentrations. Consistent with identification of the CYP2A6 as the high affinity pathway in humans from the literature and the large variability in metabolism for MTBE and other substrates in humans for this enzyme, several scenarios were modeled that allowed for different dose metric estimates at relevant environmental exposures that would be difficult to design experimentally. They show that TBA concentration in the blood varies to a much greater extent than does MTBE when metabolism is varied.

Keywords: Physiologically-Based Pharmacokinetic Modeling, Methyl Tertiary Butyl Ether (MTBE), Tertiary Butyl Alcohol (TBA), Cytochrome P450 2A6, Exposure-Related Dose Estimating Model (ERDEM).

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*Address correspondence to Dr. Jerry N. Blancato, National Center for Computational Toxicology, Office of Research and Development, U.S. Environmental Protection Agency (B205-01); 109 TW Alexander Drive, Research Triangle Park, NC 27709, E-mail: Blancato.Jerry@epa.gov.

Introduction

MTBE is a volatile organic compound (VOC) that is used as an oxygenate additive to gasoline. Production and use of MTBE in the United States continued to grow with the implementation of the Wintertime Oxygenated Fuels program in 1992 and the Reformulated Gasoline program in 1995. According to the U.S. Department of Energy (US DOE, 2004), MTBE annual consumption peaked in 1999 at 78.9 million barrels but was estimated to still be 61.2 million barrels as of 2003. Leakage from underground storage tanks or pipelines has resulted in groundwater contamination. Recreational boating activities, and release and then precipitation of non-combusted MTBE from gasoline have resulted in surface water reservoir contamination. MTBE travels rapidly to and through surface or underground water and once below the surface does not appear to adsorb to soil particles or readily degrade. MTBE is perceived by many to have an unpleasant terpene-like odor and solvent-like taste at relatively lower concentrations than many other common chemicals. Given that the potential for human exposure is quite large from ground-water contamination, the potential for toxicity through this route of exposure has been an important consideration.

MTBE is readily absorbed by both oral and inhalation exposure routes and is widely distributed throughout the body. A growing body of evidence describes the uptake and disposition of MTBE in humans from short-term exposures. These studies have primarily focused on the exploration of the MTBE kinetics as well as that of its metabolite tertiary butyl alcohol (TBA) after inhalation exposure (Prah et al., 2004, 1994; Dekant et al., 2001; Lee et al., 2001; Amberg et al., 1999; Nihlén et al., 1999, 1998; Buckley et al., 1997; Cain et al., 1996, 1994; Pekari et al., 1996; Johanson et al., 1995). Some have included examination of other metabolites such as 2-methyl-1,2-propanediol (MPD) and 2-hydroxyisobutyrate (HBA) (Dekant et al., 2001; Nihlén et al., 1999), and two manuscripts contain information concerning other routes of exposure to MTBE (Prah et al., 2004; Dekant et al., 2001). The above studies have been conducted with relatively few healthy subjects (average of 6 with a maximum of 14 for any study) that were primarily male (82%). In some studies subjects were sedentary during exposure (Prah et al., 2004; Dekant et al., 2001; Lee et al., 2001; Amberg et al., 1999; Buckley et al., 1997; Cain et al., 1996, 1994; Pekari et al., 1996; Prah et al., 1994) or examined under conditions of light exercise (Nihlén et al., 1999, 1998a; Johanson et al., 1995). One study exposed subjects concurrently to gasoline and MTBE (Lee et al., 2001). MTBE concentrations used in the studies have ranged from those commonly found from gasoline exposure to those similar to occupational exposure limits. Exposure durations have ranged from 15 minutes to 4 hours.

Physiologically based pharmacokinetic (PBPK) models are often used to estimate and predict the toxicologically relevant dose of foreign compounds in the body. These models are also particularly suited for performing extrapolations between different routes of exposure and between different species. PBPK models are formulated with species-specific anatomical, physiological, chemical-specific, and biochemical parameters, but are dependent upon accurate estimates of the governing parameters. Chemical-specific parameters include thermodynamic properties such as solubility and biochemical parameters such as the metabolic transformation rates within the tissues of the body.

Several reports and publications exist regarding the pharmacokinetics and PBPK models for MTBE and its major metabolite TBA (Cal/EPA, 1999; Rao and Ginsberg, 1997; Borghoff et al., 1996). These models can predict area under the curve (AUC) for concentration in the liver, blood, and kidney for MTBE and TBA. A PBPK model published by Prah et al. (2002) was built to describe a specific data set determined from clinical studies, especially after dermal exposure to water containing contaminants. The different models each describe accurately at least one data set in either humans or rats. On the other hand, the Rao and Ginsberg (1997) model and the model used here are compared across several data sets and for both species, and were not designed just to describe one data set. In any case, the different published PBPK models for MTBE are similar in structure. Basically, the body is represented as several explicit compartments (i.e. specifications in the model representing different tissues or groups of tissues) including the liver, kidney, and fat. Other organs are included in "lumped" compartments called "highly-perfused" and "slowly-perfused." This structure is similar to that of the models used in the past for many other VOCs. The model is a basic flow-limited model. Therefore, the disposition of a chemical throughout the body is governed by blood flow to specific compartments, the volume of those compartments, and the partitioning of the chemical in those compartments. The disposition of the chemical throughout the body is further governed by the partitioning between organs

and blood, partitioning between blood and air, the blood flows to organs, ventilation rates, absorption rates, metabolic rates, and elimination rates.

The PBPK models of Borghoff et al. (1996) and Rao and Ginsberg (1997) assume that human metabolism is similar to that of the rat, since the values were scaled up from rat values. In rats, MTBE metabolism is described by a low- and high-K_M pathway, with the cytochrome P450 isoform, CYP2E1, thought to be responsible for the low-K_M or high-affinity pathway (Brady et al., 1990). The PBPK model from Licata et al. (2001) estimated human *in vivo* metabolism values using human microsomal data, where maximum metabolic rate was assumed to be proportional to body weight to the 0.75 power, and the affinity constant was assumed to be the same as the *in vitro* value. The Licata PBPK model, which is based on the Borghoff PBPK model, also assumed that there were two metabolic pathways defined by a low metabolic rate coupled with a high affinity (lower K_M value), and another distinct pathway characterized by a higher metabolic rate constant coupled with a lower affinity (higher K_M) value. These authors suggested that the low-affinity and high-capacity pathway correlates with CYP2A6, while the high-affinity and low-capacity pathway correlates with CYP2E1. In addition, Hong et al. (1999) and Le Gal et al. (2001) have shown that it is the CYP2A6 isoform that metabolizes MTBE in human microsomal preparations. The catalytic efficiency is low but data are consistent with it being responsible for the low-K_M pathway. In two banks of human liver microsomes, Le Gal et al. (2003) reported high correlations between metabolism of MTBE, nicotine, coumarin, and immunoquantification of CYP2A6. CYP2A6 inhibition studies of human microsomal preparations and regression analyses suggested that there may be some portion of MTBE that is not metabolized exclusively by CYP2A6. As to what P450 isoform may be responsible for that activity, there are inconsistent data with CYP2E1 and CYP3A4, both being described as likely candidates (Le Gal et al., 2003, 2001; Hong et al. 1999).

In this study, we incorporate biological updates to the existing models (Cal/EPA, 1999; Rao and Ginsberg 1997; Borghoff et al., 1996) in our own model, and compare model predictions with experimental rodent data. We configured our model for humans and assigned values for MTBE metabolism using both scaled metabolic parameters derived from rodents, as was done by Rao and Ginsberg (1997), and using extrapolated human microsomal values. We then compared our predictions to experimentally derived values. Finally, given the importance that differences in MTBE metabolism may make for potential susceptibility to its toxicity, we conducted an impact analysis of variability in metabolism for dose metrics that may be useful for development of an inhalation Reference Concentration (RfC) or an oral Reference Dose (RfD) to protect from MTBE-induced adverse, noncancer health effects.

Methods

Selection of human database.

For the study of toxicity, there is a paucity of oral data (the exposure of interest due to ground-water contamination). Short-term inhalation data are available for humans that can be used to test the performance of PBPK models used for route-to-route extrapolations. These studies vary with respect to levels and durations of exposure so that a common exposure metric is needed as a basis for comparison between studies. If Haber's law holds true, then the product of level and duration of exposure should provide a common expression of "total exposure" for such purposes. The available human studies (Prah et al., 2004, 1994; Dekant et al., 2001; Lee et al., 2001; Nihlén et al., 1999, 1998; Buckley et al., 1997; Cain et al., 1996, 1994; Pekari et al., 1996; Johanson et al., 1995) were examined for accuracy, potential differences in protocol, and consistency in the relationship between "total exposure" levels to peak blood concentrations of MTBE and TBA. While overall there was a consistent relationship between MTBE "total exposure" and peak blood values, a few results did not demonstrate this consistent relationship, and were excluded for comparison with modeling results. Dekant et al. (2001) reported MTBE blood levels for their 40-ppm exposure group that were both inconsistent with other values in their study and those from other studies. No explanations were provided for the discrepancies. Similarly, TBA blood concentrations after 3 ppm MTBE exposure were much lower in the study of Prah et al. (2004) than in other studies. In the Cain et al. (1996, 1994) studies, the estimates of TBA blood concentrations were uninterpretable due to demonstrated measurement error. Peak MTBE blood levels had increased uncertainty due to inconsistent standardization within that same study. The human data used to test model performance are listed by study and exposure group in Table 1.

Structure of the PBPK model.

PBPK models describe the mass balance of materials within and between various tissue compartments. Global models typically describe each tissue as having a vascular compartment, an extracellular tissue space compartment, and an intracellular compartment. When the transfer of substances across the capillary and cellular membrane is very rapid compared to the perfusion rate of the tissue, the three compartments can be collapsed into one homogeneous compartment depicting the whole organ. In these cases, the model is called a flow-limited model and the ratio of concentration between the blood and the organ (partition coefficient) is governed by the solubilities in each tissue and metabolic processes within the organ. A detailed mathematical description of these formulations and their reduction to flow-limited conditions is found in Blancato and Bischoff (1992). Some organs are not believed to be involved in metabolizing or binding the compound of interest and are not sites of toxicity. For these organs, the partitioning (between tissue and blood), the organ volume, and blood flow govern the disposition of the chemical, and the organs are lumped together in the "highly-perfused" or the "slowly-perfused" compartments. The thermodynamic basis for lumping compartments is discussed in Bernareggi and Rowland (1991). Fat is usually not lumped with other organs because of its comparatively large volume and properties that can favor partitioning of a lipophilic substance to it from the blood, thus acting as a large "sink." For the MTBE PBPK model, fat was modeled as a separate organ (tissue). The liver and kidney were also modeled as individual organs because they are organs of metabolism and/or elimination and may be specific target sites of toxicity.

As previously discussed, each compartment is described as "well-stirred", with instantaneous and homogeneous dispersion of the chemical into the compartment once it arrives to the organ *via* the blood flow. An equation based on the principle of mass balance was written for each compartment in the model. These equations were then solved (or integrated) simultaneously for the tissue concentration of interest. The AUC for the solved tissue concentration time course can be obtained by integrating this solution once again.

Table 1. Relationship between total inhalation exposure and peak MTBE or TBA

Parameter	Exposure value[a]	Study and mean value	Mean for exposure group
Relationship of total exposure for Inhalation (ppm*min) and mean (± variation) peak TBA blood levels (µmol TBA/L)	1.39 – 5 ppm	Buckley et al. (1997)/ Prah et al. (1994) n = 2 (middle of range) = 548 ppm*min Dekant et al. (2001) n = 6 (mean) = 415 ppm*min Lee et al. (2001) n = 6 (middle of range) = 288 ppm*min Prah et al. (2003) n =14 (mean) = 980 ppm*min	417 ppm*min (without Prah)
	25 ppm	Johanson et al. (1995)/ Nihlén et al. (1998)[b] n =10 (mean) = 429 ppm*min, 468 ppm*min (average 449 ppm*min) Pekari et al. (1996) n = 4 (mean) = 545 ppm*min	497 ppm*min
	40, 50, or 75 ppm	Johanson et al. (1995)/ Nihlén et al. (1998)[b] n =10 (mean) = 480 ppm*min, 500 ppm*min (average 490 ppm*min) Nihlén et al. (1999)[b] n = 4 (mean) = 500 ppm*min Dekant et al. (2001) n = 6 (mean) = 426 ppm*min Pekari et al. (1996) n = 4 (mean) = 529 ppm*min	496 ppm*min

[a]*via* exposure concentration not dose surrogate; [b]With light exercise.

The differential equation describing the disposition of the compound within the organ (or compartment) was as follows:

$$\frac{dC_i}{dt} = Q_i(C_a - \frac{C_i}{R_i})\frac{1}{V_i} \qquad (1)$$

where,

- C_i = the concentration in organ "i",
- t = time,
- C_a = the concentration in the arterial blood,
- Q_i = the arterial blood flow to organ "i",
- R_i = the equilibrium ratio (i.e., partition coefficient) of concentrations in organ "i" and the venous blood exiting from that tissue
- V_i = the organ volume.

For metabolizing organs (e.g. the liver), the equation was modified by inclusion of a term to account for the metabolism of the compound and to account for total mass balance. For the liver, the following equation was used:

$$\frac{dC_l}{dt} = (Q_l(C_a - \frac{C_l}{R_l}) - RAM)\frac{1}{V_l} \qquad (2)$$

where,

- C_l = the concentration in liver,
- Q_l = the arterial flow into the liver,
- C_a = the concentration in the arterial blood,
- R_l = partition coefficient in the liver,
- V_l = liver volume,
- RAM = rate of amount metabolized, usually described as a saturable enzymatic process, or first order. Other forms, such as second order, can also be used when appropriate.

The PBPK model for MTBE was configured through EPA's PBPK modeling framework, ERDEM (Exposure-Related Dose Estimating Model) (Blancato et al., 2004, 2002) and is shown in Figure 1. In ERDEM, the model is defined on the basis of the species and the chemicals to be modeled. ERDEM allows the user to customize a given model at the compartment level and contains a menu-driven database requiring the user to input species- and chemical-dependent variables, and the dose metrics of interest for output. For MTBE, the compartments selected for modeling were arterial and venous blood, liver, kidney, brain, fat, and rapidly perfused and slowly perfused tissues. An important difference between this approach and others presented in the PBPK literature for MTBE (Cal/EPA, 1999; Rao and Ginsberg, 1997; Borghoff et al., 1996) is that our PBPK model was configured to have explicit pulmonary compartments through ERDEM. Therefore, the ERDEM-based PBPK models have included the complete equation for lung chemical transport, instead of using a steady-state approximation used previously by other authors. Accordingly, a lung tissue compartment is available to account for the passage of chemicals from the alveolar space through the pulmonary interstitial tissue and then into the blood. The selected parameters were set so as to simulate rapid transport from the alveolar space into the blood or the reverse for exhalation, and are believed to be equivalent to the steady-state approximation used in previous descriptions.

Simulations of the ERDEM-configured model were accomplished through the Advanced Continuous Simulation Language (ACSL). For the rat, our model was configured with parameter values taken from Borghoff et al. (1996) and Rao and Ginsberg (1997) that are listed in Table 2. Two processes for the metabolism of MTBE were used (i.e., a low- and high-affinity pathway). For humans, our model used the parameter values summarized in Table 3. These values were primarily adapted from Rao and Ginsberg (1997) and used rat partition coefficients for humans. The basal metabolic rate constants were scaled according to the body weight to 0.7^{th} power and the physiologic constants were obtained from various literature sources.

A sensitivity analysis was conducted with the rat model to determine the key parameters that impacted model predictions of AUC for MTBE in blood. Twenty-two parameters were sensitive to at least one of the exposures chosen (100 ppm and 8,000 ppm MTBE for 6 hours/day of exposure for 48 and 96 hours, respectively, or 100 and 4,000 mg/kg/day for 48 and 96 hours, respectively) and are listed in Table 4. Using the same exposure paradigm and parameters, a preliminary Monte Carlo analysis was performed using 1,000 iterations for rats and humans. The variances of those parameters were established from literature values, when available, and from best judgment based on scientific knowledge. For humans, the baseline case (as described in Table 3) was varied assuming a

coefficient of variation or percent of the mean of 20 to 50 percent for the 24 parameters varied in the analysis. Only the metabolism parameters and first-order absorption rate constant were varied by 50 percent.

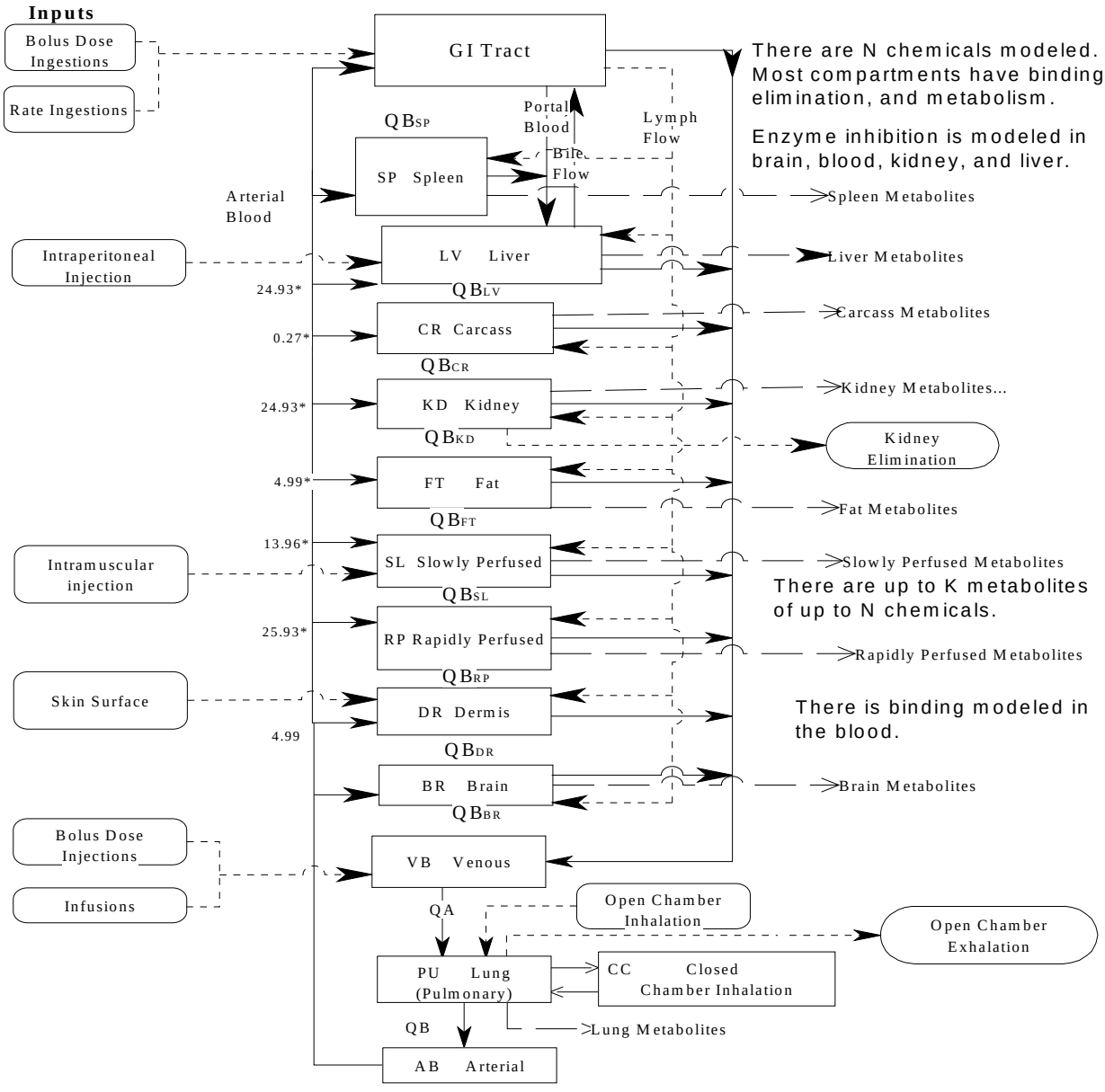

Figure 1. Schematic representation of ERDEM (Exposure Related Dose Estimating Model), version 4.1.1. This is a general schematic representation of PBPK modeling using ERDEM with example flow rate values for each organ.

Table 2. PBPK model parameters for the rat.

Parameter	Value by Rao and Ginsberg (1997)	Value by Borghoff et al. (1996)	Value used in this model	Comment
Body weight (kg)	0.25	0.18–0.28	Range	
Organ volumes as fraction of body weight (L)[1]				
Liver	0.04	0.04	0.04	
Fat	0.07	0.07	0.07	
Kidney	0.004	0.007	0.004	
Slowly perfused	0.75	0.75	0.75	
Richly perfused	0.04	0.043	0.04	
Brain	0.006	N/A	0.006	Borghoff model had no explicit brain compartment
Cardiac output (L/h) (CO)	5.38	2.52–3.92	5.38	Value used for this model was assumed to be 0.25-kg rat; varied according to body weight
Alveolar vent (L/h)	5.38	2.52–3.92	5.38	Value used for this model was assumed to be 0.25-kg rat; varied according to body weight
Organ blood flows as fraction of cardiac output (L/h)[2]				
Liver	0.24	0.25	0.24	
Fat	0.05	0.07	0.05	
Rapidly perfused	0.33	0.3	0.33	
Slowly perfused	0.19	0.15	0.19	
Brain	0.04	N/A	0.04	Borghoff model had no explicit brain compartment
Partition coefficients for MTBE				
Blood to air	11.5	11.5	11.5	
Liver to blood	1.29	1.18	1.29	
Fat to blood	10	10.05	10	
Rapid to blood	1.29	3.11	1.29	Borghoff value reported by California EPA
Slow to blood	0.57	0.565	0.57	Borghoff used value calculated for muscle

Table 2. PBPK model parameters for the rat (cont.)

Parameter	Value by Rao and Ginsberg (1997)	Value by Borghoff et al. (1996)	Value used by EPA	Comment
Kidney to blood	3.11	3.11	3.11	Borghoff value for heated kidney
Brain to blood	1.29	N/A	1.29	Borghoff model had no explicit brain compartment
Lung tissue to blood	N/A	N/A	0.5	
Partition coefficients for TBA				
Blood to air	481	481	481	
Liver to blood	0.83	0.83	0.83	
Fat to blood	0.4	0.4	0.4	
Slow to blood	0.40	1.01	0.4	Borghoff used value calculated for muscle
Kidney to blood	1.13	1.13	1.13	
Brain to blood	0.83	N/A	0.83	Borghoff model had no explicit brain compartment
MTBE metabolism parameters				
V_{max1} MTBE (mg/kg/h)	0.4	0.74	0.4	
V_{max2} MTBE (mg/kg/h)	44	9.3	44	
K_{M1} (mg/L)	0.2	0.123	0.2	
K_{M2} (mg/L)	110	21.77	110	
TBA metabolism parameters				
V_{max} TBA (mg/kg/h)	4	4.78	8	In this model tested values from 4–8
K_M (mg/L)	28	33	28.8	

[1]Organ volume percent was 100% in the ERDEM model used. This Table is concise and is not showing the percent values for the lung (0.0066), arterial blood volume (0.0276) and venous blood volume (0.0558). Once these fractions are added to the ones listed, the total becomes 100%.
[2]The kidney flow percent values used in ERDEM is 0.15, and the total flow rate volume is 100%.

Table 3. PBPK model parameters for the human.

	Value for 70 kg body weight			
Body volume	70 L			
Alveolar ventilation rate	350 L/h rest; 700 L/h exercise[1]			
Cardiac output	378 L/h rest; 425 L/h exercise[2]			
First-order absorption rate constant stomach to portal	$(1.60\ h^{-1})$ [3]			
			Partition coefficient to blood	
Compartment	**Volume % body weight**	**Blood flow % cardiac output**	**MTBE**	**TBA**
Arterial blood	2.25[4]			
Brain	2[5]	9[9,3]	0.82[3]	0.87[3]
Dermis	9[6]	4.8[10,3]	2.32[3]	
Fat	18.5[6]	4.8[10,12]	6.5[3]	0.41[3]
Kidney	0.4[5]	16[9,3]	2.02[3]	1.18[3]
Liver	2.85[6]	24[10,12]	0.82[3]	0.87[3]
Static lung: arterial blood	1.4[6]		1.5	3
Static lung: air			17.7[3]	462[3]
Rapidly perfused tissue	4.56[7]	25.4[11]	0.82[3]	0.87[3]
Slowly perfused tissue	54.5[8]	16[10]	0.37[3]	0.42[3]
Venous blood	4.5[4]			
Metabolism parameters	**Michaelis-Menten constant (mg/L)**	**Maximum velocity of metabolism (mg/H/kg[a])**		
MTBE to TBA for low affinity case (K_{M2})	110[3]	44[3]		
MTBE to TBA for high affinity case (K_{M1})	0.2[3]	0.4[3]		
TBA elimination: liver	28[3]	4[3]		
TBA elimination: kidney		1st Order Elimination: 0.5 hr-1 [3]		

[1] Corley RA et al. (1990) – values at rest.
[2] Arms and Travis (1988) – human cardiac output range from 4.6 to 6.5 L/min. The value used in the table corresponds to 6.3 L/min (value at rest).
[3] Rao and Ginsberg (1997).
[4] Igari et al. (1983) – from Brown et al. (1997), p. 460.
[5] ICRP (1975) – from Brown et al. (1997), p 418.
[6] ICRP (1975) – dermis: 3.7%, fat: 21.4%, liver: 2.6%, lungs without blood: 0.76%.
[7] Difference from 100% and all other organs.
[8] ICRP (1975) – values: muscle + bone percentage added.
[9] Brown et al. (1997) – Table 27 for brain: 12%; kidney: 19%.
[10] Brown et al. (1997) – Table 27.
[11] Calculated as the difference between 100% and the sum of all other organ percentage flows.
[12] Fisher et al. (1998).

Table 4. Parameters affecting MTBE AUC in rat blood.

Body volume	Liver to venous blood partition coefficient – TBA
Fat volume	Slowly perfused tissue to venous blood partition coefficient – MTBE
Slowly perfused tissue volume	Slowly perfused tissue to venous blood partition coefficient – TBA
Activity period alveolar ventilation rate	1st Michaelis-Menten constant for MTBE metabolism
Cardiac output	1st V_{max} for liver saturable MTBE metabolism
Fat blood flow rate - % cardiac output	2nd V_{max} for liver saturable MTBE metabolism
Liver blood flow rate - % cardiac output	Elimination constant corresponding to Michaelis-Menten constant
Fat to venous blood partition coefficient – MTBE	Elimination constant corresponding to V_{max} constant
Kidney to venous blood partition coefficient – MTBE	Static lung to arterial blood partition coefficient – MTBE
Kidney to venous blood partition coefficient – TBA	Static lung to air partition coefficient – MTBE
Liver to venous blood partition coefficient – MTBE	1st order absorption flow rate constant - stomach to portal blood - MTBE

PBPK model performance test.

Numerous comparisons between model predictions and available data were made using our model configured for rats (Table 2) and for humans (Table 3). For rats, observed data for peak MTBE or TBA concentrations in plasma (Miller et al., 1997; [unpublished] Bio-Research Labs, 1990a, b, c) and blood (Amberg et al., 1999; Savolainen et al., 1985) were used. The model predictions of Cal/EPA (1999), Rao and Ginsberg (1997), and Borghoff et al. (1996) were also compared to the same experimental data and compared to those of our model. The human model was run under the exposure conditions of each study listed in Table 1. The mean values for peak MTBE and TBA blood concentrations from all of the available data sets were compared to model predictions with exceptions previously described for the Dekant et al. (2001), Cain et al. (1996), and Prah et al. (2004) data sets.

To examine the issue of whether MTBE and TBA blood concentrations or AUCs increased in a linear fashion with exposure concentration, a 1-hour simulation of inhalation exposure at 1 ppm was run and compared with predictions at higher concentrations. Under completely linear conditions, the AUCs or blood concentrations predicted from the model should increase at the same rate as increased exposure.

Impact analyses of metabolism.

An impact analysis was conducted to assess the effects of modulation in enzyme parameters used in the human model as reported in Table 3. An extrapolation was performed from the human microsomal data and both rat and human metabolic parameters were modified to assess potential differences between humans.

To estimate *in vivo* values from microsomal values, an average value of microsomal protein of 33 mg/g was used based on the inter-individual variability study of human microsomal protein and hepatocellularity per gram of liver by Wilson et al. (2003). Using assumptions of an average liver weight of 1.5 kg, the V_{max1} of 0.81 pmol/ min-mg protein, a molecular weight of 88.4 g/mole for MTBE and 33 mg protein/g liver, the V_{max1} was transformed to 210 mg/hr and converted to 10.7 mg/hr/kg by assuming the human body weight of 70 kg adjusted to the 0.7^{th} power. Similarly, the V_{max2} was transformed to 918 m/hr or 47 mg/hr/kg. K_{M1} was 22 mg/L and K_{M2} was 256 mg/L, as given by Le Gal et al. (2003, 2001) as the average for 3 human liver microsomal preparations. Although there are no human data for the metabolism of TBA, an assumption was made for this analysis that the large difference in MTBE and TBA metabolism in rats is consistent across species. Thus, the K_M and V_{max} for TBA metabolism for the scenario using extrapolations of human microsomal data were adjusted based on the relationship between rat microsomal TBA metabolism and human microsomal MTBE metabolism (i.e., 120-fold greater K_M and 7-fold greater V_{max} for TBA metabolism in comparison with MTBE metabolism) (Le Gal et al., 2001, 2003; Cederbaum and Cohen, 1980). The adjusted K_M for TBA was 2,640 mg/L and V_{max} is 75 mg/hr/kg.

Nine scenarios were used to test the effects of change in metabolism parameters. All other parameters in the human model were kept the same as those reported in Table 3. Exposure concentrations tested were at environmental exposure levels. MTBE and TBA levels in the blood, the 4-hour AUC and metabolism in the liver were estimated. Scenario #1 used the rat metabolism of Rao and Ginsberg (1997). Scenario #2 used extrapolated parameters from human microsomal preparations of Le Gal et al. (2003, 2001). Scenario #3 had no metabolism by any pathway. The

V_{max} for the high-affinity pathway was varied 24-fold in both directions using rat metabolic parameters (i.e., scenarios # 4 and #6) and extrapolated human microsomal parameters (i.e., scenarios # 5 and #7). The high-affinity pathway was assigned a V_{max} of zero to see the proportion of metabolism that is coming from the low-affinity pathway (i.e., scenario # 9). The effects of induction of the low-affinity pathway, was also examined (i.e., scenario #8).

The human model was configured with all parameters from Rao and Ginsberg (1997) with and without the substitution of human microsomal metabolic parameters for those of the rat to test the effect on model predictions by parameters other than metabolism.

RESULTS

Human data sets.

MTBE blood concentrations peak and rapidly decrease with cessation of MTBE exposure while TBA levels steadily increase, remain high for several hours after cessation of exposure, and then slowly decline with an average half-life of 8 to 10 hours for most experimental paradigms in humans. The available human data are grouped by exposure concentration (i.e., 1- to 5- vs. 40-, 50- or 75-ppm exposure levels) and the "total exposure" (i.e., the concentration in ppm needed to give peak MTBE or TBA blood levels of 1 μmol/L), and are presented in Table 1. In general, the relationships between peak MTBE or TBA concentration and exposure concentration were linear with dose (i.e. the low- and high-exposure groups had the same slopes as the measured per unit of exposure). Greater variability existed for the data in the lower exposure group.

PBPK model testing and performance for the rat.

The PBPK model predictions by the Borghoff et al. (1996), Rao and Ginsberg (1997), and our model as well as the existing experimental data for MTBE and TBA maximum blood (Amberg et al., 1999; Savolainen et al., 1985) and plasma (Miller et al., 1997; Bio-Research Laboratories, 1990a, b, c) concentrations in the rat are shown in Table 5. Although all the PBPK rat models cited in Table 5 are basically derivations of the Borghoff et al. (1996) model with slight parameter modifications, they gave different results. The Borghoff et al. (1996) and Rao and Ginsberg (1997) models tended to give similar results for predicted MTBE blood concentrations while our model and that of Cal/EPA gave estimates that were 70 to 95% higher at 400- and 8,000-ppm exposure concentrations. For predictions of TBA concentration, all four models gave different results depending on the exposure concentration that varied by as much as 3-fold.

Borghoff et al. (1996), Rao and Ginsberg (1997), and Cal/EPA (1999) compared their PBPK model predictions for MTBE and TBA blood concentrations with those for plasma labeled as blood. Experimental data for the oral route of exposure were also labeled as 40 rather than the 33.5 and 42.7 mg/kg exposure concentrations used for male and female rat, respectively (Miller et al., 1997). In addition, Miller et al. (1997) only presented mean plasma concentrations so that the variation in the experimental data is unknown. The relationships between blood and plasma MTBE and TBA concentrations are difficult to determine without accurate measurement of the red blood cell to plasma partition coefficient and the hematocrit. The exposure concentrations and durations differ between the limited available data for plasma and blood in the rat as shown in Table 5. There are no blood oral data for comparative purposes. Thus, a data gap exists that increases the uncertainty of the rodent PBPK modeling results due to lack of data to test performance.

Savolainen et al. (1985) reported similar peak MTBE blood concentrations after 2 or 6 weeks of 50-, 100-, or 300-ppm exposures. TBA concentrations were similar to those of MTBE at 2 weeks of exposure but consistently higher than those of MTBE after 6 weeks. Both Amberg et al. (1999) (at a single 40-ppm MTBE exposure) and Savolainen et al. (1985) (after 6 weeks of 50 ppm exposure) reported higher TBA to MTBE blood ratios (i.e., 3- to 5-fold higher TBA than MTBE). All four PBPK models listed in Table 5 predicted that TBA concentrations will be higher than those of MTBE in rat blood after 400-ppm exposure (i.e., a range of 2- to 5- fold greater). Peak plasma data also indicated TBA concentrations can be higher than those of MTBE. Miller et al. (1997) reported 2.5-fold higher plasma concentrations for TBA compared with those of MTBE at the lowest concentration tested while at the higher concentrations MTBE and TBA concentrations were approximately the same.

Table 5. PBPK model performance test for the rat.

Model Predictions	**MTBE C_{max} (mg/L)**	**TBA C_{max} (mg/L)**
Borghoff et al. (1996)[a]		
Inhalation:		
400 ppm (6-hour exposure, male)	Blood: 12.9	Blood: 54.9
8000 ppm (6-hour exposure, male)	Blood: 305	Blood: 251
Oral:		
40 mg/kg (single oral exposure, male)	Blood: 8.8	Blood: 18.0
400 mg/kg (single oral exposure, male)	Blood: 133	Blood: 81.8
Rao and Ginsberg (1997)[b]		
Inhalation:		
50 ppm (6-hour exposure, male)	Blood: 1.44	Blood: NA
100 ppm (6-hour exposure, male)	Blood: 2.96	Blood: NA
300 ppm (6-hour exposure, male)	Blood: 9.52	Blood: NA
400 ppm (6-hour exposure, male)	Blood: 12.0	Blood: 38.0
8000 ppm (6-hour exposure, male)	Blood: 290	Blood: 464
Oral:		
40 mg/kg (single oral exposure, male)	Blood: 11.0	Blood: 15.0
400 mg/kg (single oral exposure, male)	Blood: 129	Blood: 110
Cal/EPA (1999)[c]		
Inhalation:		
400 ppm (6-hour exposure, male)	Blood: 19.4	Blood: 99.3
8000 ppm (6-hour exposure, male)	Blood: 499	Blood: 728
Oral:		
40 mg/kg (single oral exposure, male)	Blood: 6.01	Blood: 13.0
400 mg/kg (single oral exposure, male)	Blood: 70.8	Blood: 167
Our model[b]		
Inhalation:		
4.5 ppm (4-hour exposure, male)	Blood: 0.104	Blood: 0.558
38.7 ppm (4-hour exposure, male)	Blood: 1.47	Blood: 3.53
400 ppm (6-hour exposure, male)	Blood: 19.5	Blood: 39.8
8000 ppm (6-hour exposure, male)	Blood: 582	Blood: 255
Oral:		
40 mg/kg (single oral exposure, male)	Blood: 12.5	Blood: 14.3
400 mg/kg (single oral exposure, male)	Blood: 153	Blood: 94.0
Experimental Data	**MTBE C_{max} (mg/L)**	**TBA C_{max} (mg/L)**
Miller et al. (1997)/BioResearch Laboratories (1990)[d]		
Inhalation:		
408 ± 26.8 ppm (6-hour exposure, male[e])	Plasma: 15.7	Plasma: 38.4
8038 ± 461 ppm (6-hour exposure, male[e])	Plasma: 557	Plasma: 482
Oral:		
33.5 ± 0.54 mg/kg (single oral exposure, male[f])	Plasma: 17.2	Plasma: 10.0
42.7 mg/kg (single oral exposure, female[d])	Plasma: 11.2	Plasma: 8.9
420 ± 9.6 mg/kg (single oral exposure, male[g])	Plasma: 124	Plasma: 50.3
388 mg/kg (single oral exposure, female[d])	Plasma: 115	Plasma: 48.8
Savolainen et al. (1985)[h]		
Inhalation:		
50 ppm (6-hour exposure, 2 weeks)	Blood: 0.800	Blood: 0.371
100 ppm (6-hour exposure, 2 weeks)	Blood: 1.59	Blood: 1.26
300 ppm (6-hour exposure, 2 weeks)	Blood: 5.92	Blood: 4.96
50 ppm (6-hour exposure, 6weeks)	Blood: 0.972	Blood: 2.82
100 ppm (6-hour exposure, 6 weeks)	Blood: 2.12	Blood: 6.08
300 ppm (6-hour exposure, 6 weeks)	Blood: 5.83	Blood: 11.2
Amberg et al. (1999)[i]		
Inhalation:		
4 ppm (4-hour exposure, male and female)[j]	Blood: 0.203	Blood: 0.215
40 ppm (4 hour exposure, male and female)[j]	Blood: 0.522	Blood: 2.72

[a] Mean body weight = 180-280 g for male rats
[b] Mean body weight = 250 g for male rats
[c] Mean body weight = 215 g for male rats
[d] Mean body weight = 129-150 g for female rats
[e] Mean body weight = 186 g for male rats
[f] Mean body weight = 198 g for male rats
[g] Mean body weight = 199 g for male rats
[h] Mean body weight = started at 211 g and was 407-420 by week 15 for male rats (assume 30 g weight gain in 2 weeks gives weight of 241g at 2 weeks and 301 at 6 weeks)
[i] Mean body weight = weight ranges were 210-240 g for males and 190-200 for female rats
[j] Background levels for MTBE were not detected but for TBA were 0.126 mg/L
NA = not available

The Rao and Ginsberg (1997) PBPK model over-predicted peak MTBE blood concentrations in the rat by a factor of 2 in comparison with the 50-, 100-, and 300-ppm exposure concentration data reported by Savolainen et al. (1985). Rao and Ginsberg (1997) did not report model predictions of TBA for comparative purposes. In comparison to the rat data of Amberg et al. (1999), our model under-predicted MTBE blood concentrations by a factor of 2 at the 4.5-ppm exposure concentration and over-predicted them by a factor of 2.8 at the 38.7-ppm level. For TBA, peak blood concentrations were over-predicted by a factor of 2.6 at 4.5-ppm and by only 30 % at the 38.5-ppm exposure concentrations without correction for background TBA levels. A comparison of our model blood concentration predictions with rat plasma data reported by Miller et al. (1997) showed predicted MTBE blood concentration were higher by 25% and 5% at 400- and 8,000-ppm exposure concentrations, respectively. Although we cannot be certain without proper measurements of key parameters, blood concentrations higher than plasma are consistent with partitioning favoring red blood cells over plasma. Our model predicted TBA blood concentrations to be 4% greater and 47 % less for plasma than those reported for the same data.

PBPK model testing and performance for the human.

Comparisons of predicted human peak MTBE and TBA concentrations using our model, as configured in Table 3, with available human data sets are presented in Tables 6 and 7. The modeled outputs are always within 3-fold of the reported values (means) and in most cases within less than 2-fold. The biggest differences between reported data and model outputs were observed for TBA. It should be noted that model predictions are being compared to means and the standard deviations as reported and not to data for individuals.

Table 6. Comparison of human model predictions for MTBE with reported values (means)

Study	Reported MTBE peak (μmol/L)	Modeled MTBE peak (μmol/L)
Dekant et al., 2001(4 ppm, 4 h exposure)	1.9	0.8
Dekant et al., 2001(40 ppm, 4 h exposure)	6.7	10
Cain et al., 1994,1996 (1.7 ppm, 1 h exposure)	0.18–0.23	0.24
Buckley et al., 1997/Prah et al., 1994 (1.39 ppm, 1 h exposure)	0.093 (males) 0.16 (females)	0.19
Johanson et al., 1995/Nihlén et al., 1998 (50 ppm, 2 h exposure)	13	14.9
Johanson et al., 1995/Nihlén et al., 1998 (25 ppm, 2 h exposure)	6.5	7.2
Johanson et al., 1995/Nihlén et al., 1998 (5 ppm, 2 h exposure)	1.3	1.3
Pekari et al., 1996 (25 ppm, 4 h exposure)	11	5.9
Pekari et al., 1996 (75 ppm, 4 h exposure)	29	19.8
Lee et al., 2001(1.7 ppm, 15 min exposure)	0.045– 0.12	0.13
Prah et al., 2004 (3 ppm, 1 h exposure)	0.26	0.42

Sensitivity and Monte Carlo analyses.

For the rat model, a sensitivity analysis showed that 22 of the 41 original input parameters affected MTBE blood concentration. For the subsequent Monte Carlo analysis of the human model, two more parameters (i.e., static lung to arterial blood partition coefficient for TBA and static lung to air partition coefficient for TBA) were varied. The Monte Carlo analysis should reveal the uncertainty and variability (or stochastic behavior) of each dose metric due to variation of parameter estimates and not to other uncertainties in the model. Under the conditions of the analysis, venous blood MTBE AUC varied by approximately 4-fold between the 95th to the fifth percentile and by approximately 9-fold for TBA. However given the high exposure concentrations and the limited number of iterations, the results for humans are of limited value. The greater variation in TBA blood concentration than that of MTBE identified metabolism for more specific analyses.

Metabolism impact analyses.

The impact analyses of variation in metabolic parameters in the human model on dose metrics of potential interest for risk assessment are presented in Table 8. In all of the impact analyses, all other parameters were kept the same as those listed in Table 3 with the exception of metabolism. The effect of changes in metabolic parameters on the linearity of the relationship between predicted peak MTBE or TBA blood and exposure concentrations was examined using either the rat metabolic parameters of Rao and Ginsberg (1997) or the extrapolated values from human microsomal preparations. The results of multiplication of predicted blood concentrations at 1 ppm with the magnitudes of exposure increases were compared with modeled values at higher exposures. The variations from linearity of model predictions were compared to those of the experimental database. For human data, a comparison of slopes between the low- vs. high-exposure groups (i.e., 1- to 5- vs. 40- to 75-ppm) in Tables 6 and 7 shows an 8% increase in MTBE peak blood and 20% decrease in TBA peak blood concentration from the low- to high-exposure group. Using human microsomal values (i.e., scenario #2), our model predicted that there is no variation from linearity in the relationship between peak MTBE or TBA blood and 1-ppm to 40-ppm exposure concentrations. However at the same exposure concentrations, use of rat metabolic values (i.e., scenario #1) gives a 23 % increase in MTBE

Table 7. Comparison of human model predictions for TBA with reported values (means)

Study	Reported TBA peak (μmol/L)	Modeled TBA peak (μmol/L)
Dekant et al., 2001 (4 ppm, 4 h exposure)	2.6	1.6
Dekant et al., 2001(40 ppm, 4 h exposure)	21.8	10.4
Cain et al., 1994,1996 (1.7 ppm, 1 h exposure)	N/A	
Buckely et al., 1997/Prah et al., 1994 (1.39 ppm, 1 h exposure)	male: 0.13 female: 0.14	0.15
Johanson et al., 1995 /Nihlén et al., 1998 (50 ppm, 2 h exposure)	12.5	8.8
Johanson et al., 1995 /Nihlén et al., 1998 (25 ppm, 2 h exposure)	7	5.4
Johanson et al., 1995 /Nihlén et al., 1998 (5 ppm, 2 h exposure)	ND	
Pekari et al., 1996 (25 ppm, 4 h exposure)	16	7.5
Pekari et al., 1996 (75 ppm, 4 h exposure)	34	16.1
Lee et al., 2001 (1.7 ppm, 15 min exposure)	2–4 h post-exp.: 0.065	0.05
Prah et al., 2004 (3 ppm, 1 h exposure)	3 h post-exp.: 0.19	0.33

peak blood and 44% decrease in TBA peak blood concentrations in comparison to the linear case. MTBE blood AUCs (24 hours) follow the same pattern as the peak MTBE blood levels while the amount metabolized at 24 hours and maximum TBA concentration in the liver follows the same pattern as the maximum TBA concentration in the blood. Thus, in terms of predictions of linearity, our model performs better using human microsomal values.

Differences in model predictions of the absolute magnitude of the peak MTBE blood levels using rat metabolic parameters or human microsomal values are small at these exposure concentrations. Peak MTBE blood levels are about 30% higher under scenario #1 than scenario #2 at the 1-ppm exposure level with both scenarios giving predictions within 5% of each other at the 40-ppm level. For the MTBE AUC values, the differences between scenarios #1 and #2 are about 30 % at 1-ppm and 15 % at 40-ppm exposure concentrations.

A comparison was made between model predictions using rat or human microsomal values and those in which the V_{max} for both enzyme pathways were set to zero and metabolism was eliminated. Under scenario #1 without metabolism, peak MTBE levels were increased by 40%, 38%, and 23% for 1-, 5-, and 40-ppm exposure levels, respectively. The 24-hour MTBE blood AUC was increased by 50%, 47%, and 36% at the same exposure levels. Under scenario #2, regardless of exposure, the peak MTBE levels are only increased by 20% and MTBE blood AUCs increased by 25% by eliminating all metabolism. Therefore using rat or human microsomal metabolic parameters, the effects of metabolism on MTBE levels in the blood were not greater than 50% and thus MTBE blood levels are relatively refractory to MTBE metabolism under these conditions at environmental exposure levels. However, changes in metabolism did have a greater impact on peak TBA blood levels as shown in Table 8.

Under scenario #2, the contribution of the low affinity pathway to changes in MTBE blood concentration from metabolism appeared to be only about 33 % at all exposure concentration levels as shown by scenario #9. However, induction of the low-affinity pathway is possible if the enzyme responsible for the low affinity pathway is CYP2E1. Increases in the low-affinity pathway by a 24-fold increase in V_{max2} (i.e., scenario 8) decreased MTBE peak blood and MTBE AUC (24 hrs) by about 50%. The effect on TBA blood concentration from induction of this pathway was much greater (i.e., TBA peak blood concentrations were increased 2.8-fold).

Unless induced, the high-affinity pathway is responsible for 66% of the reduction of MTBE in the blood by metabolism and, assuming it is CYP2A6, is highly variable between individuals. By both increasing (scenarios #4 and #5) and reducing (scenarios #6 and #7) V_{max1} 24-fold for the high affinity pathway, a range of MTBE and TBA predicted concentrations can be estimated that serves as a reasonable test of the effects of metabolic variation on model predictions. Under these conditions, changes to scenario #1 produced a range of 3.1-fold for peak MTBE concentrations and 3.7-fold for MTBE AUCs (24 hrs) for 1- to 40-ppm exposure concentrations. The corresponding range for maximal TBA concentration in the blood was 5.1- to 5.6-fold. For scenario #2 these changes produced a range of 2.6-fold for peak MTBE concentrations and 3.2-fold for MTBE AUC (24 hrs) for 1- to 40-ppm exposure conditions. The corresponding range for maximal TBA concentration in the blood was 9-fold. If the low-affinity pathway is also induced by 24-fold, the range in TBA blood concentration is increased to 13-fold. If the activity of the high-affinity pathway is set to zero rather than being decreased 24-fold, as is the case for an individual with a nonfunctional CYP2A6 enzyme, that range increases to 15-fold.

Examination of all parameters.

Along with model structure, there were differences in parameters reported by Rao and Ginsberg (1997) and those used in our model for humans. Table 9 shows predicted peak MTBE blood concentrations at 5- and 50-ppm exposure concentrations using our model structure but with different input parameters. Using all of the parameters in Table 3, our model produced slight over-predictions of MTBE blood concentrations compared to one of the more robust human databases (Johanson et al. 1995/ Nihlén et al. 1998). When all the input parameters reported from Rao and Ginsberg (1997) for humans were applied to our model, it did a worse job at predicting peak MTBE concentrations. Substitution of human microsomal parameters for those of the rat did not improve the performance of the model under these conditions.

Table 8. Impact analysis: effects of changes in metabolic parameters on MTBE dose-metrics.

Metabolic parameters K_M (mg/L) V_{max} (mg/hr/kg) [1]	Exposure level (ppm)	MTBE peak concentration 24 hours post exposure (mg/L)	MTBE AUC at 24 hours post exposure (mg/L-hr)	Maximum TBA concentration in liver at 4 hours (mg/L)	Maximum TBA concentration in blood (mg/L)	Amount metabolized at 24 hours post exposure (mg)
Scenario #1						
MTBE	1	0.0171	0.0829	0.0300	0.0315	2.36
K_{M1} = 0.2; V_{max1} = 0.4	5	0.0899	0.433	0.139	0.147	11.3
K_{M2} = 110; V_{max2} = 44	40	0.885	4.24	0.696	0.770	68.5
TBA	400	9.99	51.6	3.97	4.37	423
K_M = 28; V_{max} = 4						
Scenario #2						
MTBE	1	0.0233	0.123	0.0156	0.0170	1.21
K_{M1} = 22; V_{max1} = 10.7	5	0.117	0.615	0.0780	0.0857	6.07
K_{M2} = 256; V_{max2} = 47	40	0.937	4.94	0.615	0.678	48.1
TBA	400	9.66	50.7	5.38	6.06	443
K_M = 2,640; V_{max} = 75						
Scenario #3						
MTBE	1	0.0289	0.164	0	0	0
K_{M1} = 22; V_{max1} = 0	5	0.144	0.823	0	0	0
K_{M2} = 256; V_{max2} = 0	40	1.15	6.59	0	0	0
TBA	400	11.5	65.9	0	0	0
K_M = 2,640; V_{max} = 0						
Scenario #4						
MTBE	1	0.00799	0.0357	0.0546	0.0574	0.0145
K_{M1} = 0.2; V_{max1} = 9.6	5	0.0400	0.179	0.273	0.287	18.5
K_{M2} = 110; V_{max2} = 44	40	0.326	1.45	2.19	2.30	147
TBA	400	6.37	25.3	14.3	16.0	1170
K_M = 28; V_{max} = 4						
Scenario #5						
MTBE	1	0.0102	0.0463	0.0551	0.576	3.40
K_{M1} = 22; V_{max1} = 257	5	0.0509	0.231	0.276	0.288	17.0
K_{M2} = 256; V_{max2} = 47	40	0.407	1.85	2.20	2.30	136
TBA	400	4.13	18.7	21.9	22.9	1350
K_M = 2,640; V_{max} = 75						
Scenario #6						
MTBE	1	0.0246	0.132	0.0106	0.0113	0.957
K_{M1} = 0.2; V_{max1} = 0.017	5	0.124	0.663	0.0513	0.0550	4.68
K_{M2} = 110; V_{max2} = 44	40	1.00	5.38	0.379	0.408	35.2
TBA	400	10.2	54.7	3.53	3.82	326
K_M = 28; V_{max} = 4						
Scenario #7						
MTBE	1	0.0268	0.148	0.00570	0.00641	0.474
K_{M1} = 22; V_{max1} = 0.45	5	0.134	0.742	0.0285	0.0320	2.37
K_{M2} = 256; V_{max2} = 47	40	1.07	5.94	0.227	0.255	18.9
TBA	400	10.8	59.6	2.17	2.46	184
K_M = 2,640; V_{max} = 75						
Scenario #8						
MTBE	1	0.0133	0.0622	0.0455	0.0476	2.95
K_{M1} = 22; V_{max1} = 10.7	5	0.0664	0.311	0.227	0.238	14.7
K_{M2} = 256; V_{max2} = 1128	40	0.532	2.49	1.82	1.90	118
TBA	400	5.35	25.0	18.1	18.9	1180
K_M = 2,640; V_{max} = 75						
Scenario #9						
MTBE	1	0.0270	0.150	0.00517	0.00582	0.432
K_{M1} = 22; V_{max1} = 0	5	0.135	0.750	0.0258	0.0291	2.16
K_{M2} = 256; V_{max2} = 47	40	1.08	6.00	0.206	0.232	17.3
TBA	400	10.8	60.1	2.01	2.28	170
K_M = 2,640; V_{max} = 75						

[1] V_{max1} and K_{M1} are metabolic constants for the high-affinity pathway; V_{max2} and K_{M2} are metabolic constants for the low-affinity pathway.

For TBA, the experimental database is much more limited and variable at low levels of human exposure. However our model, as configured in Table 3, predicted peak TBA blood levels to be within 60% of those measured experimentally at 1.7- to 4-ppm exposure concentrations and generally under-predicted TBA levels from 40 to 100% at the 40- to 50-ppm exposure levels. Using all of the parameters of Rao and Ginsberg (1997) increased our model predictions of peak TBA blood levels by 30%. Substitution of human microsomal metabolism parameters for those of the rat further increased TBA blood predictions by 53%.

Thus, our model modifications and parameters improved the ability of the model to predict the MTBE levels in the blood. Predictions of TBA levels against human data are difficult due to the limitations and variability in the database but seem to indicate that our modifications make them marginally worse. However, it should be cautioned that because of the paucity of data, comparisons are only possible with a few endpoints for TBA and are mostly peak concentrations. Typically, model comparisons with time course disposition data are preferred. Such data are not available for the studies indicated in Tables 6 and 7.

Discussion

An important aspect in assessing the performance of a PBPK model is how well it fits several data sets. As a first step, it is important to understand and characterize that data and to choose measured endpoints for comparison with predicted results. The accuracy and precision of the methods used in each study and the apparent reproducibility of results must be examined so that such judgments can be made. Variation of results reported from differing studies may reflect human variability of response, the effects of differing exposure protocols, or differences in the accuracy and precision of measured values in each study.

For the MTBE human database, specific studies had design features that added to the strength of their results. Lee et al. (2001) tested their subjects twice. Nihlén et al. (1999) repeated their protocol after 3 years with the same subjects. Variations between exposure conditions and results for Nihlén et al.'s 1998 and 1999 studies were small, thereby increasing the confidence that variation in response between subjects is a reflection of human variation under those exposure conditions. Nihlén et al. (1998, 1999) measured respiration rather than assuming standard

Table 9. Comparisons between model structures and mean peak levels for Johansen/Nihlén human data.

Model configuration	MTBE exposure concentration	% difference in modeled vs experimental peak concentration of MTBE
Parameters: As given in Table 3	5 ppm	Same
	50 ppm	+ 13%
Parameters: All parameters as given by Rao and Ginsberg (1997)	5 ppm	+42%
	50 ppm	+57%
Parameters: All parameters as given by Rao and Ginsberg (1997) with exception of human microsomal values substituted for rat metabolism	5 ppm	+53%
	50 ppm	+53%

values, which gives added confidence in their uptake estimates.

Alternatively, there were weaknesses in specific studies, common limitations of studies of this type, and inconsistent results that can be put into context by examination of the database as a whole. Some studies were more limited by the extent data were presented (e.g., Cain et al., 1996, 1994; Pekari et al., 1996), reported results that were either not consistent with other values in their study (Dekant et al., 2001) or with those from other studies (Dekant et al., 2001, Prah et al., 2004), or had demonstrated measurement error (Cain et al., 1996, 1994). All studies used small numbers of healthy subjects that were relatively homogeneous in their physical characteristics, so inferences about the total magnitude of human variation for the whole population are limited. However, an examination of the available human data for short-term exposure to MTBE reveals that while there was large variability in the relationship between total exposure and peak MTBE levels between individual subjects, the mean values were similar across studies and exposure level groups.

As a starting point for development of our PBPK model, we examined the PBPK models of Borghoff et al. (1996) and Rao and Ginsberg (1997). While both models are based on similar structure, there were differences that provided the rationale for selection of one over the other. These differences were important in the decision needed to establish the current version of the PBPK model presented in this paper. While Borghoff et al. (1996) determined parameters for the specific rats used in their experiments, Rao and Ginsberg (1997) determined several key parameters from comparisons to data from several different rat studies and thus would be more representative of the greater heterogeneity of rats used in the toxicity studies. The Rao and Ginsberg (1997) values used for cardiac output and alveolar ventilation in rats are also similar to values EPA has used in other simulations.

For metabolic parameter determination, Borghoff et al. (1996) used an approach designed by Andersen et al. (1987), the gas uptake method. In summary, animals were put in a closed exposure chamber with various initial concentrations of MTBE and declining concentrations of MTBE were carefully monitored. The PBPK model was specifically configured to have an additional compartment, the exposure chamber. Physiologic and thermodynamic parameters were fixed in the model after being determined separately from laboratory measurements (partition coefficients) or from literature sources (relative organ volumes, blood flows). The model was then run, and the metabolic parameters were adjusted to fit the family of changing concentration curves for MTBE in the exposure chambers. Each group of animals was exposed to a different initial concentration. Thus, a time versus concentration-curve was generated for each initial concentration.

Although this is a powerful technique, it has some limitations. One endpoint—in this case the MTBE concentration in the exposure chamber—is used to fit four metabolic parameters (V_{max1}, V_{max2}, K_{M1}, and K_{M2}). It is possible that several different combinations of values could result in equally good fits. In such cases, there is no way to easily distinguish which set of values is best. This is crucial, because although several combinations of parameter values could fit the chamber data, they could each result in different estimates of an internal toxicologically relevant dose. The likelihood that such a problem exists is diminished by the fact that several different initial concentration level experiments are performed. Theoretically, this should reduce the number of possible combination values that will fit the data.

In contrast, Rao and Ginsberg (1997) used a different approach from Borghoff et al. (1996) to estimate rat metabolic parameters. They fit their model to blood MTBE and TBA concentrations resulting from 6-hour constant inhalation exposure studies at 8,000-ppm MTBE. From these data, they determined the best fit values for the metabolic rate constants for MTBE biotransformation to TBA. The values Rao and Ginsberg (1997) used for the subsequent biotransformation of TBA were taken directly from Borghoff et al. (1996). The question as to which type of inhalation exposure is more sensitive to the actual determination of metabolic parameters needs to be addressed in the toxicology-related PBPK literature, and is considered a suggestion for future research.

A summary of the differences between previous PBPK models for MTBE reveals the improvements made to the current model. For example, Rao and Ginsberg (1997) used the tissue- and blood-to-air partition coefficients of Borghoff et al. (1996), but one notable exception was the slowly-perfused tissue-to-blood partition coefficient for TBA. Borghoff et al. (1996) used muscle tissue to determine the tissue-to-air value in the laboratory, and then used this muscle value in their model to represent the coefficient for the slowly-perfused tissue. Our present model incorporated a skin compartment, as described in Rao

and Ginsberg (1997), who calculated the skin permeation coefficients according to U.S. EPA (1992b). There is no skin compartment in the model of Borghoff et al. (1996). For the physiologic and anatomic parameters (e.g., compartment volumes, blood flows), Borghoff et al. (1996) stated they used values from the literature. Rao and Ginsberg (1997) determined the partition coefficients according to the methods of McDougal et al. (1990). For the physiologic and anatomic parameters (e.g., compartment volumes, blood flows), Borghoff et al. (1996) stated they used values from the literature. However, in the summary table, they stated that the alveolar ventilation and cardiac output "were estimated independently and held fixed." Further examination indicates that these values were determined from the literature.

Borghoff et al. (1996) adjusted the volume by trying to account for the high solubility of TBA in body water. Alternatively, Rao and Ginsberg (1997) adjusted the kidney-to-blood partition coefficient for TBA by fitting the model against actual blood data. This value was reported as 1.01 by Borghoff et al. (1996), whereas the final adjusted value reported by Rao and Ginsberg (1997) was 0.4. However, both groups of investigators have noted that the PBPK model adequately predicts TBA concentrations only when some modifications are made separately to each model.

With regard to binding of MTBE or TBA to alpha 2_u globulin and its effect in distribution to the kidney, our model made use of the same assumptions as Rao and Ginsberg (1997), and Borghoff et al. (1996), and treated distribution of MTBE to the kidney of the male and female rat in the same way. Therefore, the effects of binding of MTBE to proteins in the male rat kidney on cumulative exposure to the kidney as described by Kohn and Melnick (1999) were not incorporated in these models.

An important feature of PBPK model development is the consistency of its predictions with experimental data from different data sets. For the human model, Rao and Ginsberg (1997) compared their model to a time course of concentration and optimized the model against those values. For our human model, we did a similar analysis and obtained similar values (results not shown). However, we further optimized the model against multiple data sets, but not using time course of concentration in all cases as they were not available for most of the studies referenced and used here. Instead, we configured the model to match reported values of peak concentration and an AUC cited in several studies. While this is not typically done for optimization, given the paucity of time-course data and the interest in these two dose metrics, we considered this to be a viable alternative. Under ideal conditions, optimization would be done with more data. However, it should be noted that changing our model so that it produced predictions that were more consistent with "tails" of the concentration profile had little appreciable effect on the predictions of maximum MTBE concentrations or 24–hour AUCs. Thus, fitting the model against these robust dose metrics was considered a reasonable approach for this case.

In a separate optimization procedure, Licata et al. (2001) combined the inhalation time courses from three human exposure groups from two data sets (Amberg et al., 1999 and Cain et al., 1996) and estimated *in vivo* values for the metabolic constants in both pathways. The regression results for the first pathway were: normalized V_{max} = 34.5 µmol/kg-hour with a K_M = 61.4 µmol/L. These authors compared simulations obtained using these values with venous blood MTBE data obtained during constant inhalation at 4 and 40 ppm (held constant at 4 hours), and a one hour constant inhalation exposure at 1.7 ppm. Visual examination of the published plots suggests that reasonable fits for peak and post exposure values were obtained at the lowest and highest exposure concentrations (1.7 and 40 ppm), but that simulations underpredicted the blood MTBE concentrations at the intermediate exposure concentration of 4 ppm. The type of impact analyses performed in this report on the effects of metabolic variation in MTBE and TBA predicted values, were not done by Licata et al., 2001.

Uncertainty related to model structure is related to several factors. First, all available literature reports ascribe all metabolism to the liver. No metabolism within the kidney or lung (or respiratory tract) has been depicted in any of the models. These tissues may be likely candidates to metabolize MTBE to TBA. The likely impact of metabolism at these sites could be substantial if the model does not account for it and the most appropriate dose metric were to be a metabolite of MTBE (TBA or other) in the respiratory tract or kidney itself. There could be more variation in the AUC, peak concentrations, and elimination of minor metabolites, especially in extra-hepatic tissues. Redistribution of P450 metabolism is more realistically seen in organs other than the liver. Metabolism, regardless of tissue, was considered a clearance process for MTBE. Any metabolism in the lung or

kidney would increase the clearance of MTBE in blood. Due to lack of data regarding this issue, it is unknown at this time how to account quantitatively for extra-hepatic metabolism of MTBE for this effort. A variability analysis can take into account the potential effects of possible extra-hepatic metabolism but more data would be needed in order to adjust for the presence of both hepatic and extrahepatic metabolism in the model. Our PBPK model is structured to account for extrahepatic metabolism should data become available.

Although comparisons of model predictions with experimental results (i.e., MTBE peak concentrations) published in several different human data sets show those predictions to be within 3-fold of the published values (means), it should be noted that these comparisons are based on relatively small samples of adult male humans or rats. The true extent of variation in a larger, more heterogeneous human population would be greater and in many cases could be considerable.

Variation in MTBE metabolism may be an important determinant in variation of toxicological response, but the role of either MTBE or its metabolites in its toxicity has not been established. There is less data for comparison of TBA levels in humans and PBPK models do not predict TBA levels in the blood as well as those for MTBE. It is assumed that MTBE blood levels will be proportional to overall metabolism over time so that it is a reasonable surrogate for toxicity regardless of whether metabolite or parent compound is the active agent. A sensitivity analysis of the rat PBPK model showed that about 20 model parameters affect MTBE blood concentrations and that variation in the predicted TBA blood concentrations were greater than that of MTBE. The impact analysis of our human model also demonstrated that metabolism changes would affect TBA blood concentrations to a greater extent than those of MTBE. If TBA is the agent responsible for some or all of the toxic effects of MTBE, then the variation in human response could be much greater than that predicted by MTBE blood concentrations.

In addition, the alteration of other parameters in our human model was shown to affect model predictions of MTBE and TBA concentrations. Gender, age, and other factors may affect these parameters. PBPK models have not yet been developed for MTBE that take into account differences between children and adults. There must be development of databases for pharmacokinetic differences (e.g., metabolism) and pharmacodynamic considerations. For some infants and children, assumptions concerning magnitude of variation may not be adequate.

A number of other solvents are efficiently metabolized in the liver resulting in metabolic variation between individuals primarily reflecting differences in blood flow to the liver and not in the affinity or capacity of their metabolic enzyme systems. This does not appear to be the case for MTBE. The metabolism of MTBE appears to be limited by the activity of metabolic enzymes and can therefore be called "enzyme limited" rather than by the amount of MTBE presented to the liver through the blood. Thus, potential differences in response due to route of exposure may be minimal. A "first pass effect" would be expected, as would blood "flow limitation" of metabolism, if the liver was efficient in removal of MTBE from the blood. Hence, orally administered MTBE would have a greater chance of MTBE metabolism by the liver than MTBE exposure via inhalation. However, the production of TBA from MTBE metabolism is limited by the low efficiency of enzymes for this substrate. In addition, available human data shows TBA levels in the blood appear to take several more hours to reach their peak concentration after cessation of inhalation exposure and to reach a level similar to that of MTBE (Dekant et al., 2001; Lee et al., 2001; Nihlén et al., 1999, 1998a; Buckley et al., 1997; Pekari et al., 1996; Johanson et al., 1995).

As important as the assignment of metabolic parameters is to model predictions of MTBE and TBA concentrations, uncertainty exists for both use of extrapolated rat values and with procedures used for extrapolation of human values from *in vitro* systems (i.e., as was done for extrapolations of microsomal human liver data). This is especially true for enzymes that are membrane bound. It is not clear how differently these enzymes behave in *in vitro* systems where they are often released from these membranes. It should be noted, however, that it is not clear how values can be extrapolated from experimental animals to humans. The most common method is to extrapolate based on a power of the body weight (0.7 in this case) and this method inherently assumes that, pharmacokinetically, a human behaves like a large rodent. This assumption does not take into account possible species differences in enzymes, isoenzymes, and different levels of polymorphism, etc., between species. Extrapolation between species for substrates of CYP2A6, like MTBE, can be difficult. Le Gal et al.

(2003) point out that while CYP2A6 catalyzes coumarin 7-hydroxylation in humans as the major pathway of metabolism, this activity is negligible in rat and mouse liver microsomes. Similarly, CYP2A6 is the major enzyme involved in nicotine metabolism in humans but animal studies cannot be extrapolated to man because of species-related differences in the catalytic activities of the CYP2A enzymes.

Identification of the enzyme responsible for MTBE metabolism is not only important for determining how well MTBE is metabolized but also how variable humans may be in that activity. The variation reported between subjects in enzymatic activity for MTBE metabolism is consistent with the database for coumarin and nicotine, whose metabolism is primarily by CYP2A6, as correlated with MTBE metabolism, but with a much lower K_M than MTBE. Iyer and Sinz (1999) report that the liver microsomal bank considered to be most representative of the human population, a IIAM bank of 164 individuals, gave a 113-fold range in metabolism at a coumarin concentration of 0.1 mM. Le Gal et al. (2001, 2003) reported that inter-individual variability in CYP2A6 activity for coumarin activity was 9-fold but for MTBE was 24-fold (104 to 2,493 pmol/min/mg, n=11 at 0.5 mM or 2 times the K_M) in 11 subjects. Licata et al. (2001) used two separate procedures to obtain metabolic estimates using human data. In the first group of calculations, the authors scaled values obtained *in vitro* from human microsomes using an *in vitro* vial equilibration technique (a scaled down version of the *in vivo* gas uptake approach). With this approach, they obtained a maximum value for a normalized V_{max} of 117.8 µmol/kg-hour and a K_M of 93.9 µmol/L for the high capacity, low-affinity pathway associated with CYP2A6. The minimum values for the same pathway were: normalized V_{max} = 4.5 µmol/kg-hour and K_M = 48.0 µmol/L. The estimated mean values were: normalized V_{max} = 33.8 µmol/kg-hour with a standard deviation of 34.1 µmol/kg-hour, and a K_M = 61.7 µmol/L with a standard deviation of 12.4 µmol/L. The 25-fold difference between the maximum and the minimum metabolic values for the extrapolated results is consistent with the variation in 11 subjects for MTBE microsomal metabolism. If such a relationship between variation in coumarin and MTBE metabolism would hold true in a larger liver bank such as that of the IIAM, then the variation in MTBE metabolism may be even larger than that reported for coumarin in the general population.

Raunio et al. (2001) report CYP2A6 activity to have variability between ethnic groups with several alleles having no activity. Homozygous individuals for these alleles will have no metabolic capability. Le Gal et al. (2001, 2003) reported that even with the large variability in MTBE metabolism from a limited number of human livers, no individual was CYP2A6 deficient as measured by ability to metabolize nicotine and coumarin. The 24-fold increase or decrease from the average value for the V_{max1} used for the impact analyses was only 8-fold higher than the top and 3-fold lower than the bottom range of values in the 11 subjects studied in Le Gal et al. (2001, 2003). Thus, the adjustments used in the impact analyses were a reasonable bound for examining the effects changed in MTBE metabolism in the broader population. However, the true extent of metabolic variation in the whole population is unknown.

Summary

We have described the development and application of a PBPK model based on multiple published data sets and other published models. While our model had significant limitations, it performed well within those limitations and its performance was improved over existing models. We used the model for several purposes. One of those uses is the examination of impacts of changes in metabolic capacity on the potential dose metrics that may be used in an assessment of risk from MTBE exposure. In doing so, we help characterize and identify uncertainties associated with assumptions regarding the toxicology of MTBE. Our model is a cost-effective and available tool for such an examination that would not be otherwise possible using conventional laboratory and clinical approaches. It illustrates that models can be developed with certain limits and used appropriately within those limits. In summary, models are reasonable depictions of what is known using available information and data. The acceptability of the model, given its limitations and its supporting data, is dependent on its intended use and a good understanding of those limitations and imperfections. Regardless of its use, model limitations, background biology, and data must be clearly articulated and transparent.

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References

Amberg A, Rosner E, Dekant W. (1999) Biotransformation and kinetics of excretion of methyl-tert-butyl ether in rats and humans. Toxicol Sci; 51:1–8.

Anderson ME, Clewel HJ, Gargas ML, et al. (1987) Physiologically based pharmacokinetics and the risk assessment process for methylene chloride. Toxicol Appl Pharmacol; 87:185–205.

Bernareggi A, Rowland M. (1991) Physiologic modeling of cyclosporine kinetics in rat and man. J. Pharamcokineti Biopharm 19(1) 21- 50

Bioresearch Laboratories. (1990a) Pharmacokinetics of methyl tert-butyl ether (MTBE) and tert-butyl alcohol (TBA) in male and female Fischer-344 rats after administration of MTBE by the intravenous, oral and dermal routes. Report No. 38842 Bioresearch Laboratories Ltd, Senneville, Quebec, Canada.

Bioresearch Laboratories. (1990b) Mass balance of radioactivity and metabolism in male and female Fischer-344 rats after intravenous, oral and dermal administration of 14C-methyl tertiary-butyl ether. Report No. 38843 Bioresearch Laboratories Ltd., Senneville, Quebec, Canada.

Bioresearch Laboratories. (1990c) Pharmacokinetics of methyl tert-butyl ether (MTBE) and tert-butyl alcohol (TBA) in male and female Fischer-344 rats after single and repeated inhalation nose-only exposures to MTBE. Report No. 38844 Bioresearch Laboratories Ltd., Senneville, Quebec, Canada.

Blancato JN, Bischoff KB. (1992) "The application of Pharmacokinetic Models to Predicted Target Dose." In Health Risk Assessment Through Dermal and Inhalation Exposure and Absorption of Toxicants, Wang, R.G.M, Knaak, J.B., Maibach, H. I. editors CRC Press. p 31 - 46.

Blancato JN, Power FW, Brown RN, and Dary CC. (2004) Exposure Related Dose Estimating Model (ERDEM) for Assessing Human Exposure and Dose. U.S. Environmental Protection Agency. EPA/600/R-04/060, December 2004.

Blancato JN, Power FW, Wilkes CR, et al. (2002) Integrated Probabilistic and Deterministic Modeling Techniques in Estimating Exposure to Water-borne Contaminants: Part 2: Pharmacokinetic Modeling in Proceedings of Indoor Air 2002 International Conference.

Borghoff SJ, Murphy JE, Medinsky MA. (1996) Development of a physiologically based pharmacokinetic model for methyl tertiary-butyl ether and tertiary butanol in male Fischer-344 rats. Fundam Appl Toxicol: 30:264–275.

Brady JF, Xiao F, Ning SM, et al. (1990) Metabolism of methyl-tertiary butyl ether by rat hepatic microsomes. Arch Toxicol 64:157–160.

Brown RP, Delp MD, Lindstedt SL, et al. (1997). Physiological parameter values for physiologically based pharmacokinetic models. Toxicol Ind Health. 13:407-84.

Buckley TH, Prah JD, Ashley D, et al. (1997) Body burden measurements and models to assess inhalation exposure to methyl tertiary butyl ether (MTBE). J Air Waste Manage Assoc; 47:739–752.

Cain WC, Leaderer BP, Ginsberg GL, et al. (1994) Human reactions to brief exposures to methyl tertiary-butyl ether (MTBE). Report submitted by the John B. Peirce Laboratory, New Haven CT on 3/2/94 to the Methyl Tertiary Butyl Ether Task Force.

Cain WC, Leaderer BP, Ginsberg GL, et al. (1996) Acute exposure to low-level methyl tertiary-butyl ether (MTBE): human reactions and pharmacokinetic response. Inhal Toxicol; 8(1):21–48.

EPA (California Environmental Protection Agency). (1999). Public health goal for methyl teriary butyl ether (MTBE) in drinking water. Office of Environmental Health Hazard Assessment. Cal EPA, Sacramento, CA.

Cederbaum AI, Cohen G. (1980) Oxidative demethylation of t-butyl alcohol by rat liver microsomes. Biochem Biophys Res Commun; 97:730–736.

Dekant W, Bernauer U, Rosner E, et al. (2001) Biotransformation of MTBE, ETBE, and TAME after inhalation or ingestion in rats and humans. Health Effects Institute research report: metabolism of ether oxygenates added to gasoline, Number 102. Cambridge, MA.

Hong J-Y, Wang Y-Y, Bondoc FY, et al. (1999a) Metabolism of methyl tert-butyl ether and other gasoline ethers by human liver microsomes and heterologously expressed human cytochromes P450: identification of CYP2A6 as a major catalyst. Toxicol Appl Pharmacol; 160:43–48.

Iyer KR, Sinz MW. (1999) Characterization of phase I and II hepatic drug metabolism activities in a panel of human liver preparations. Chemico-Biological Interactions; 118:151-169.

Johanson G, Nihlén A, Löf A. (1995) Toxicokinetics and acute effects of MTBE and ETBE in male volunteers. Toxicol Lett (Shannon, Ireland); 82/83:713–718.

Kohn MC, Melnick RL. (1999) A physiological model for ligand-induced accumulation of alpha2u globulin in male rat kidney: roles of protein synthesis and lysosomal degradation in the renal dosimetry of 2,2,4 - trimethyl-2-pentanol. Toxicology; 136:89–105.

Lee CW, Mohr SN, Weisel CP. (2001) Toxicokinetics of human exposure to methyl tertiary-butyl ether (MTBE) following short-term controlled exposures. J Expo Anal Environ Epidemiol; 11:67–78.

Le Gal A, Dreano Y, Gervasi PG, Berthou F. (2001) Human cytochrome P450 is the major enzyme involved in the metabolism of three alkoxyethers used as oxyfuels. Toxicol Lett; 124: 47-58.

Le Gal A, Dreano Y, Lucas D, Berthou F. (2003) Diversity of selective environmental substrates for human cytochrome P450 2A6: alkoxyethers, nicotine, coumarin, N-nitrosodiethylamine, and N-nitrosobenzylmethylamine. Toxicol Lett: 144:77-91.

Licata AC, Dekant W, Smith CE, Borghoff SJ. (2001) A physiologically based pharmacokinetic model for methyl tert-butyl ether in humans: implementing sensitivity and variability analyses. Tox Sci; 62:191-204.

McDougal JN, Jepson GW, Clewel HJ, et al. (1990) Dermal absorption of organic chemical vapors in rats and humans. Fund Appl Toxicol;14:299–308.

Miller MJ, Ferdinandi ES, Klan M, et al. (1997) Pharmacokinetics and disposition of methyl-tert-butyl ether in Fischer 344 rats. J Appl Toxicol; 17(Suppl 1):S3–S12.

Nihlén A, Löf A, Johanson G. (1998) Experimental exposure to methyl tertiary-butyl ether I. Toxicokinetics in humans. Toxicol Appl Pharmacol; 148:274–280.

Nihlén A, Sumner S, Löf A, et al. (1999) 13C2-labelled methyl tert-butyl ether: toxicokinetics and characterization of urinary metabolites in humans. Chem Res Toxicol; 12:822–830.

Pekari K, Riihimaki V, Vainiotalo S, et al. (1996) Experimental exposure to methyl-tert butyl ether (MTBE and methyl-tert-amyl ether (MTAE). In: Proceedings of the International Symposium on Biological Monitoring in Occupational and Environmental Health; September 1996; Espoo, Finland; Helsinki Institute of Occupational Health: 27–28.

Prah JD, Goldsterin GM, Devlin R, et al. (1994) Sensory, symptomatic, inflammatory, and ocular responses to and the metabolism of methyl tertiary butyl ether in a controlled human exposure experiment. Inhal Toxicol; 6:521–538.

Prah J, Ashley D, Blount B, et al. (2004) Dermal, oral, and inhalation pharmacokinetics of methyl tertiary butyl ether (MTBE) in human volunteers. Toxicol Sci; 77(2):195–205.

Prah JD, Blount B, Cardinalli FL, et al. (2002) The development and testing of a dermal exposure system for pharmacokinetic studies of administered and ambient water contaminants. J Pharmacol Toxicol Methods 47(3):189-195.

Rao HV, Ginsberg GL. (1997) A physiologically based pharmacokinetic model assessment of methyl t-butyl ether in groundwater for a bathing and showering determination. Risk Anal; 17(5):583–598.

Raunio H, Rautio A, Gullsten H, Pelkonen O. (2001) Polymorphisms of CYP2A6 and its practical consequences. Br J Clin Pharmacol; 52:357-363.

Savolainen H, Pfaffli P, Elovarra E. (1985) Biochemical effects of methyl tertiary-butyl ether in extended vapor exposure of rats. Arch Toxicol 57(4):285–288.

U.S. DOE (Department of Energy), (2004) EIA-819M Monthly Oxygenate Report: Historical. Available on the Internet at: http://www.eia.doe.gov/oil_gas/petroleum/data_publications/monthly_oxygenate_telephone_report/motr_historical.html

U.S. EPA (Environmental Protection Agency). (1992) Dermal exposure assessment: principles and applications. Interim Report. Office of Prevention, Pesticides, and Toxic Substances, Washington, DC. EPA/600/8-91/01 1B.

Wilson ZE, Rostami-Hodjegan A, Burn JL, et al. (2003) Inter-individual variability in levels of human microsomal protein and hepatocellularity per gram of liver. Br. J Clin Phamacol; 56:433-440.