Original Article

A physiologically based kinetic modeling of ethyl *tert*-butyl ether in humans—An illustrative application of quantitative structure-property relationship and Monte Carlo simulation

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ABSTRACT — Although physiologically based kinetic (PBK) modeling is informative for the risk assessment of industrial chemicals, chemical-specific input values for partition coefficients and metabolic parameters, including $V_{\max}$ and K_m are mostly unavailable; however, *in silico* methods, such as quantitative structure-property relationship (QSPR) could fill the absence. To assess the PBK model validity using necessary toxicokinetic (TK) parameters predicted by QSPR, the PBK model of ethyl *tert*-butyl ether (ETBE) as a model substance was constructed, in which the values of the partition coefficients, $V_{\max}$, and K_m of ETBE were predicted using those of the related chemicals previously reported in the literature, and toxicokinetics of inhaled ETBE were stochastically estimated using the Monte Carlo simulation. The calculated ETBE concentrations in venous blood were comparable to the measured values in humans, implying that the reproducibility of ETBE toxicokinetics in humans was established in this PBK model. The Monte Carlo simulation was used to conduct uncertainty and sensitivity analyses of the dose metrics in terms of maximum blood concentration ($C_{\max}$) and area under the blood concentration-time curve (AUC) and the estimated $C_{\max}$ and AUC were highly and moderately reliable, respectively. Conclusively, the PBK model validity combined with *in silico* methods of QSPR was demonstrated in an ETBE model substance. QSPR-PBK modeling coupled with the Monte Carlo simulation is effective for estimating chemical toxicokinetics for which input values are unavailable and for evaluating the estimation validity.

Key words: QSPR, PBK modeling, Ethyl *tert*-butyl ether, Monte Carlo simulation, Uncertainty analysis

INTRODUCTION

A physiologically based kinetic (PBK) model is a mathematical model that describes the body as respective tissue compartments connected by blood flow, and the chemical toxicokinetics absorbed through inhalation, oral, or transdermal route are estimated using mass balance equations for the chemical in each compartment. This model has been used to assess the health risks of industrial chemicals and pharmaceuticals because it can determine the relationship between external exposures and the mass of distributed chemicals or their metabolites in target tissues, and reduce toxicokinetic uncertainties during

the extrapolation of intra-species, interspecies, and inter-route doses. The Integrated Risk Information System of the U.S. Environment Protection Agency (EPA) attributed PBK models for interspecies extrapolation and route to route extrapolation, from which the Reference Concentrations and Reference Doses were derived, and ten solvents were evaluated in the model, including dichloromethane, trichloroethylene, methanol, and ethylene glycol monobutyl ether.

PBK model calculations require physiological (cardiac output, alveolar ventilation rate, tissue blood flow, and tissue volume), physicochemical (blood/air and tissue/blood partition coefficients), and biochemical parameters,

including the maximal velocity ($V_{\max}$), Michaelis affinity constant (K_m), and clearance. Although values of the physiological parameters specific to animal species and humans are available in the literature, physicochemical and biochemical parameters specific to respective substances are only available for limited chemicals. Thus, PBK models have not been widely applied for human health risk assessment of industrial chemicals. However, *in silico* methods, such as quantitative structure-property relationship (QSPR) and the category approach, in addition to *in vitro* methods using cultured cells were recently investigated to estimate these physicochemical and biochemical parameters without *in vivo* testing such as *in vitro-in vivo* extrapolation (IVIVE) that has recently attracted attention in Europe and the United States to supplement the hazard information of such chemicals. However, in the IVIVE, it is necessary to relate the concentrations in the test solution of the *in vitro* assay to the blood concentrations in organisms by appropriate PBK modeling. On the other hand, the generic type of QSPR-PBK model is believed to be useful in estimating the rapid toxicokinetics of many chemicals without such information. The partition coefficients and clearance were calculated using QSPR and *in vitro* studies, respectively, and the kinetics were predicted using PBK models in these studies (Casey *et al.*, 2018; Zhang *et al.*, 2018; Fabian *et al.*, 2019; OECD, 2020).

In recent chemical regulation, the number of chemicals that include chemicals directly exposed from the environment, leaching chemicals from package materials, and chemicals contained in household materials is increasing, but the rapid evaluation of human health risks of these chemicals is required, whereas a gradual reduction of animal studies is a global consensus from the perspectives of animal welfare and costs. Due to the difficulty in testing these chemicals in humans, it is considered that toxicology evaluation, which incorporates existing study data and studies using *in vitro* and *in silico* technologies, contributes to the refinement of risk assessments without conducting animal studies, and thus the establishment of such an approach is requested. Although measured toxicokinetic (TK) parameters prerequisite for the models are available for a limited number of chemicals, estimating or predicting these parameters using *in vitro* studies and *in silico* technologies is valid, and PBK models simulating chemical toxicokinetics are considered valuable for evaluating chemical toxicity and refinement of the risk assessment.

Ethyl *tert*-butyl ether (ETBE), a volatile liquid derived from ethanol and isobutylene, has been used in automobiles in Japan since 2007 as a fuel additive oxygen-

ates. The main route of exposure to ETBE in humans is inhalation based on its physicochemical characteristics and handling. ETBE that is once inhaled into the body is metabolized to *tert*-butanol (TBA) and acetaldehyde by cytochrome P450. TBA is further metabolized to 2-methyl-1,2-propanediol and 2-hydroxyisobutyrate. These metabolites are detected in rat and human urine (Amberg *et al.*, 2000). Salazar *et al.* (2015) and Borghoff *et al.* (2017) independently developed rat ETBE PBK models, in which the mechanism of ETBE-induced tumorigenesis in the kidney and liver was elucidated by estimating the levels of its metabolite, TBA. The model of Salazar *et al.* proposed two metabolism pathways from ETBE to TBA, whereas the model of Borghoff *et al.* proposed a single oxidative metabolic pathway from ETBE to TBA. The latter model was validated by comparing estimated blood and urine ETBE levels and TBA urine levels during or after inhalation of ETBE with the rat exposure data (Amberg *et al.*, 2000). In this model, obvious nonlinear kinetics due to metabolic saturation were predicted with inhalation of ETBE at 2000 ppm and higher concentrations. In the human ETBE PBK model, tissue volumes were calculated on the basis of weight and height of respective subjects and blood flow rates to tissues under workload, and the kinetics of ETBE and TBA metabolism were represented by hepatic clearance obtained by fitting to the experimental data measured in respective subjects (Nihlén and Johanson, 1999). For toxicology, subchronic toxicity studies (Medinsky *et al.*, 1999; Hagiwara *et al.*, 2015; U.S. EPA, 2021) and carcinogenicity studies (Saito *et al.*, 2013; Suzuki *et al.*, 2012) through the inhalation and oral routes, in addition to reproductive (Fujii *et al.*, 2010; U.S. EPA, 2021) and developmental toxicity studies (Aso *et al.*, 2014; U.S. EPA, 2021) through the oral route were conducted for this chemical. Reference Concentration and Minimum Risk Level were established by the EPA and the Agency for Toxic Substances and Disease Registry (ATSDR), respectively. In terms of environmental exposure, ETBE atmospheric emissions from production, storage, refueling, and vehicles were estimated, and the average daily exposure concentrations were calculated using a mathematical model (Makino *et al.*, 2012).

This study aims to develop a QSPR-PBK modeling technique coupled with Monte Carlo simulation and to evaluate toxicokinetics, including uncertainty, using *in silico*-estimated TK parameters. ETBE was selected as a model chemical since it had TK parameters of related chemicals required for the QSPR technique and measured TK data of the chemical that can validate the PBK model. For rational estimation of TK parameters, QSPR that uses

a dataset of TK parameters of related chemicals, which have been obtained from *in vivo* studies and are already known, is considered to be most promising, whereas sensitivity analyses that detect the most affecting parameters on TK and evaluation of uncertainties associated with the use of an estimated value instead of a measured value are fundamental. The effect of these uncertainties on the calculation results can be quantitatively evaluated using a traditional Monte Carlo approach, a Bayesian Markov chain Monte Carlo (MCMC) analysis, a stochastic response surface method, or a fuzzy simulation approach (U.S. EPA, 2006). However, there are few examples of uncertainty analysis by these Monte Carlo methods in PBK modeling. The recent interest seems to be mostly examining the variability of toxicokinetics in the human population using the MCMC method (e.g., Bois *et al.*, 2010). We attempted to develop a PBK model that could predict the human toxicokinetics of ETBE using parameters estimated by an *in silico* method, in which the partition coefficients and metabolic parameters ($V_{\max}$ and K_m) of ETBE essential for toxicokinetics estimation in humans were calculated based on information of the same category chemicals, and their uncertainties were examined as probability density functions. Furthermore, the blood concentration-time profiles and the dose metrics as maximum blood concentration ($C_{\max}$) and area under the blood concentration-time curve (AUC) were estimated with their confidence intervals using Monte Carlo simulation, and the former two were compared to the previously reported measurements (Nihlén *et al.*, 1998). Finally, the impact of the uncertainty in the partition coefficient and metabolic parameter values on the dependability of dose metrics was quantitatively evaluated and discussed.

MATERIALS AND METHODS

PBK modeling for ETBE in human

The PBK model used in this study is a Ramsey and Andersen (1984) type PBK model and consists of six tissue compartments (lung, fat, rapidly perfused tissues, slowly perfused tissues, liver, and gastrointestinal (GI) tract), and describes the kinetics of organic chemicals absorbed from the lung into the human body (Fig. 1). This type of PBK model is often applied to industrial chemicals.

The ETBE concentration change rate in non-metabolizing tissues (fat, rapidly perfused tissues, slowly perfused tissues) is described as

$$dC_i/dt = Q_i \cdot (C_a - C_i/P_i)/V_i \quad (1)$$

Fig. 1. Schematic diagram of the human PBK model. Q_a and Q_c stand for alveolar ventilation rate and cardiac output, respectively. Q_f , Q_r , Q_s , Q_h , and Q_g are for blood flow to fat, richly perfused tissue, slowly perfused tissue, liver, and GI tract, respectively. C_a and C_{ven} stand for concentration in arterial and venous blood. C_{vf} , C_{vr} , C_{vs} , C_{vh} , and C_g are for concentrations in fat, richly perfused tissue, slowly perfused tissue, liver, and GI tract, respectively. C_{in} and C_{out} refer to concentrations in the inhaled and exhaled air. K_a is for oral absorption rate constant.

where i represents fat, rapidly and slowly perfused tissues, Q_i is the blood flow in tissue i , C_a is the chemical concentration in the arterial blood, C_i is the concentration in tissue i , P_i is the tissue i /blood partition coefficient, and V_i is the volume in tissue i .

We assumed that ETBE metabolism occurred only in the liver and modeled it *via* Michaelis–Menten kinetics with $V_{\max}$ and K_m . The chemical's concentration change rate in the liver is described as follows:

$$dC_h/dt = Q_h \cdot C_a/V_h + Q_g \cdot C_g/P_{gb}/V_h - (Q_h + Q_g) \cdot C_h/P_{hb}/V_h - (V_{\max} \cdot C_h/P_{hb})/(K_m + C_h/P_{hb})/V_h \quad (2)$$

where Q_h and Q_g refer to blood flows to the liver and GI tract compartments, C_h and C_g are the chemical concentration in the liver and GI tract, P_{hb} and P_{gb} are the liver/blood and GI tract/blood partition coefficients, and V_h is the liver tissue volume.

Oral absorption is described as a first-order process. The chemical's concentration change rate in the GI tract is described as follows:

$$dC_g/dt = Q_g \cdot (C_a - C_g/P_{gb})/V_g + K_a \cdot S_{tmod}/V_g \quad (3)$$

where K_a is the oral absorption rate constant, S_{tmod} is the oral dose and V_g is the GI tract tissue volume.

The pulmonary compartment is assumed to consist of the lung tissue, the functional residual capacity (FRC), and the arterial blood, according to Perbellini *et al.* (1986). The chemical's concentration change rate in this compartment is described as follows:

$$dC_a/dt = (Q_a \cdot C_{in} + Q_c \cdot C_{ven} - Q_c \cdot C_a - Q_a \cdot C_a/P_{ba}) / (V_{lung} \cdot P_{lb} + V_{art} + FRC/P_{ba}) \quad (4)$$

where Q_a is the alveolar ventilation, C_{in} is the concentration of chemicals in the inhaled air, C_{ven} is the concentration in venous blood, Q_c is the cardiac output and P_{ba} is the blood/air partition coefficient. V_{lung} is the lung volumes, P_{lb} is the lung/blood partition coefficient, and V_{art} is the arterial blood volume.

The mixed venous blood concentration is calculated as follows:

$$dC_{ven}/dt = ((Q_h + Q_g) \cdot C_h/P_{hb} + Q_f \cdot C_f/P_{fb} + Q_r \cdot C_r / P_{rb} + Q_s \cdot C_s/P_{sb} - Q_c \cdot C_{ven})/V_v \quad (5)$$

where V_v is the venous blood volume.

The AUC of venous blood concentration from the start

of exposure to time T is calculated using the following equation:

$$AUC = \int_0^T C_{ven} \cdot dt \quad (6)$$

The model simulation code was written and executed using R version 3.6.2 (R Core Team, 2019). PBK models were solved using the package deSolve version 1.27.1 (Soetaert *et al.*, 2010) and graphs were plotted using the package ggplot2 version 3.3.0 (Wickham, 2016). The program code also includes code for Monte Carlo simulations. Based on previously released experiment data, the validity of our model's predictability has been proven. Table S1 and Fig. S1 of Supplementary material 1 illustrate validation examples.

Physiological parameters

Table 1 shows the values of the physiological parameters (cardiac output, alveolar ventilation rate, tissue volume, and tissue blood flow) used in the PBK model. These are previously reported literature values (Ali and Tardif, 1999; Filser *et al.*, 2000; Csanády *et al.*, 2003; Valentin, 2003; Benignus *et al.*, 2006; Marchand *et al.*, 2015). Also, physiological parameters at 50 W physical load are previously reported literature values (Laparé *et al.*, 1993). To calculate blood flow values, we assumed

Table 1. Physiological parameters used in the PBK model.

Parameter	Symbol	Value	
		Rest	50 W
Cardiac output, L/hr/kg ^{0.7}	Q_c	18 ^a	31.89 ^b
Alveolar ventilation, L/hr/kg ^{0.7}	Q_a	18 ^a	65.31 ^b
Blood flow as fraction of cardiac output			
Fat	Q_{fc}	0.05 ^a	0.077 ^b
Rapidly perfused tissues	Q_{rc}	0.44 ^a	0.271 ^b
Slowly perfused tissues	Q_{sc}	0.25 ^a	0.518 ^b
Liver	Q_{lc}	0.26 ^a - Q_{gc}	0.038 ^b
GI tract	Q_{gc}	0.15 ^c	0.095 ^b
Compartment volumes as fractions of body weight			
Arterial blood	V_{artc}		0.0178 ^d
Venous blood	V_{vc}		0.0533 ^c
Lung blood	V_{lungc}		0.0076 ^d
Fat	V_{fc}		0.19 ^a
Rapidly perfused tissues	V_{rc}		0.05 ^a
Slowly perfused tissues	V_{sc}		0.62 ^a
Liver	V_{lc}		0.026 ^a
GI tract	V_{gc}		0.017 ^c

^a Marchand *et al.* (2015)

^b Laparé *et al.* (1993)

^c Valentin (2003)

^d Csanády *et al.* (2003)

^e Filser *et al.* (2000)

that the cardiac output at 50 W was 56% higher than the rest cardiac output and that cerebral blood flow increased by half the amount of cardiac output increase (Furuhata *et al.*, 1996). We set the human body weight at 82 kg, which is the average weight of all subjects in the Nihlén *et al.* study (1998).

Partition coefficients and metabolic parameters

QSPR techniques were used to calculate the partition coefficients, $V_{\max}$ and K_m , required to predict the kinetics of ETBE in humans using the PBK model. Aliphatic oxygenates having intermolecular interactions similar to ETBE (mainly van der Waals forces and dipole–dipole interactions) were extracted from 181 industrial organic compounds with reported partition coefficients for humans, $V_{\max}$ and K_m . The extracted chemicals include aliphatic ethers, ketones, esters, and other non-ionized chemicals with one or more hydrogen bond acceptors. We analyzed the linear correlation between the common logarithmic values of these partition coefficients and their Henry's law constants (Henry, Pa·m³/mol) for the blood/air partition coefficients of these compounds, and for the tissue/blood partition coefficients in the liver, fat, richly perfused tissues, and slowly perfused tissues, a linear correlation of the common logarithmic values of these partition coefficients with log D (the common logarithmic value of the octanol/water distribution coefficient at pH 7.4) was also analyzed. The Henry's law constants and log D values used in the analysis were obtained from the EPI Suite (U.S. EPA, 2012) and the ICE (Integrated Chemical Environment) of the National Toxicology Program (NTP), respectively. Statistical software R version 3.6.2 was used for linear regression analysis. The selected chemicals and their human partition coefficient values, along with Henry's law constants or log D, are shown in Tables S1, S3, S5, S7, and S9 of the Supplementary material 2.

From the mean and standard error of the intercepts and slopes of the linear regression equations obtained for each partition coefficient, and Henry's law constant (166 Pa·m³/mol) or log D (1.479) of ETBE, the common logarithmic values of each partition coefficient of ETBE were set to be the following normal distributions of the mean (μ) and standard deviation (σ).

$$\mu_i = \mu_{\text{slop},i} \cdot (\log \text{Henry}_{\text{ETBE}} \text{ or } \log D_{\text{ETBE}}) + \mu_{\text{intercept},i} \quad (7)$$

$$\sigma_i = \sqrt{\sum_{i,j} SE_{\text{slop},i}^2 \cdot (\log \text{Henry}_{\text{ETBE}} \text{ or } \log D_{\text{ETBE}})^2 + SE_{\text{intercept},i}^2} \quad (8)$$

where, the subscript i denotes each partition coefficient, $\mu_{\text{slop},i}$ and $SE_{\text{slop},i}$ denote the average and standard error of the slope of each linear regression equation, respectively,

and $\mu_{\text{intercept},i}$ and $SE_{\text{intercept},i}$ denote the average and standard error of the intercept of each linear regression equation. Furthermore, the logarithmic mean and logarithmic standard deviation of the partition coefficient according to the lognormal distribution were calculated by converting the mean and standard deviation of the common logarithm to the natural logarithm.

The Free-Wilson approach was used to analyze $V_{\max}$ ($V_{\max}$ normalized with bodyweight) and K_m in chemicals consisting of hydrocarbons, halogenated hydrocarbons, and aliphatic ethers. This Free-Wilson approach has been previously applied to estimate liver clearance (Béliveau *et al.*, 2003) and $V_{\max}$ and K_m (Price and Krishnan, 2011) of aliphatic, aromatic, and halogenated aliphatic hydrocarbons in rats. However, there have been no cases of applying the Free-Wilson approach to estimating human $V_{\max}$ and K_m , and structural fragments of oxygen-containing chemicals have not been examined. Therefore, we selected the human $V_{\max}$ and K_m values of the abovementioned chemicals from the database and applied the Free-Wilson approach to derive new estimation equations. The selected chemicals and their human $V_{\max}$ and K_m values are shown in Tables S1 and S4 of the Supplementary material 3.

The Free-Wilson equation is expressed as follows:

$$\log M = \sum_i f_i \cdot CR_i \quad (9)$$

where M represents $V_{\max}$ or K_m , CR_i is the contribution of fragment i to the value of $V_{\max}$ or K_m , and f_i is the number of fragment i in the molecule. Similar to Price and Krishnan (2011), the structural fragments we used were benzene ring (BzR), benzene ring hydrogen (H_BzR), the carbon–carbon double bond (C=C), hydrogen of carbon–carbon double bond (H_C=C), methyl group (CH₃), methylene group (CH₂), methine group (CH), quaternary carbon (C), chlorine (Cl), bromine (Br), and fluorine (F), and we added the ether group (–O–) as a new structural fragment. Tables S2 and S5 of the Supplementary material 3 show the number of structural pieces found in the selected chemicals. Statistical software R version 3.6.2 was used for this multiple regression analysis. From the mean and standard error of CR_i calculated for each structural fragment, and the number of each structural fragment in ETBE, the probability density functions for the common logarithmic values of $V_{\max}$ and K_m of ETBE are set as normal distributions with the following mean (μ) and standard deviation (σ).

$$\mu_j = \sum_{i,j} f_i \cdot \mu_{i,j} \quad (10)$$

$$\sigma_j = \sqrt{\sum_{i,j} f_i^2 \cdot SE_{i,j}^2} \quad (11)$$

Table 2. Probability density function and their statistics for the uncertainties of the estimated partition coefficients and metabolic parameters of ETBE.

Parameter	Probability density function	Logarithmic mean	Logarithmic standard deviation
Partition coefficients			
Blood/air (P_{ba})	lognormal	2.75	0.380
Liver/blood (P_{hb})	lognormal	0.364	0.190
Fat/blood (P_{fb})	lognormal	2.88	0.456
Rapidly perfused tissue/blood (P_{rb})	lognormal	0.358	0.208
Slowly perfused tissue/blood (P_{sb})	lognormal	0.426	0.195
Metabolic parameters			
V_{max} , mg/hr/kg	lognormal	1.11	3.05
K_m , mg/L	lognormal	0.09	2.13

where j represents V_{max} or K_m , and $fCH3 = 4$, $fCH2 = 1$, $fC = 1$ and $f-O = 1$ for ETBE. μ_{ij} and SE_{ij} are the mean and standard error of the contribution of the structural fragment i obtained using multiple regression analysis to j , respectively.

Model simulations

In the study of Nihlén *et al.* (1998), eight male volunteers performed light exercise (50 W) in an exposure chamber, and were exposed to 5-, 25-, and 50-ppm ETBE for 2 hr and then observed for 4 hr without further exposure and exercise. Blood levels of ETBE were measured approximately 20 times during this study. Amberg *et al.* (2000) also exposed three volunteers of each gender to 4.5- and 40.6-ppm ETBE in an exposure chamber for 4 hr. They reported the maximum concentration of ETBE in each volunteer's blood and the biological half-life after exposure ended.

In our study, we evaluated the validity of the partition coefficients and metabolic parameters by calculating the concentrations using our estimated partition coefficients and metabolic parameters, and comparing them to the reported blood concentrations and biological half-lives from Nihlén *et al.* (1998) study for which concentration-time profiles were available. Furthermore, Monte Carlo simulations were applied to assess the degree of uncertainty in the estimates. Lognormal distributions of logarithmic mean and logarithmic standard deviation, as shown in Table 2 were set up for the partition coefficients and metabolic parameters as explanatory variables. Conversely, the venous blood concentration and its AUC over 6 hr were selected as the response variables. The distributions of these response variables were calculated using the Monte Carlo approach by repeatedly (1,000 iterations) sampling input values based on the distributions of individual explanatory variables. Then, sta-

tistics on the estimated venous blood concentrations and AUC were calculated. Furthermore, ETBE half-lives in the blood after exposure ended were also calculated and compared to those measured by Nihlén *et al.* (1998).

Analyses of sensitivity and uncertainty

In addition to comparing model simulations to exposure study data, we conducted uncertainty and sensitivity analyses to evaluate the effect of estimated parameters on the reliability of model estimates of dose metrics relevant to risk assessment. Overall, if the parent substance is toxic, C_{max} and AUC are appropriate dose metrics of internal exposure, and if metabolites are involved, the rate and total amount of metabolite production are appropriate dose metrics of internal exposure (U.S. EPA, 2006). In this study, we focused on C_{max} and AUC of venous blood concentrations as dose metrics of the parent substance and analyzed the sensitivity and uncertainty of partition coefficients and metabolic parameters (V_{max} and K_m) to these dose metrics according to the procedures described in the WHO IPCS (2010) document. We used the probability density functions of the statistics shown in Table 2 for the uncertainty analysis, generated a random number for only one parameter (the rest of the parameter values were fixed) and ran Monte Carlo simulations under the conditions described in the previous section. The statistics on the estimated dose metrics were obtained, and the ratio of the 95th percentile value to the median of the dose metrics was calculated. According to WHO IPCS (2010), uncertainty was considered low when this ratio was below 0.3, moderate when it was between 0.3 and 2, and high when it was greater than two.

However, to analyze the sensitivity of C_{max} and AUC to parameter values, the sensitivity ratios of incremental C_{max} and AUC (%) to a 1% increase in the parameter values were calculated. According to WHO IPCS (2010), an

Table 3. Comparison of the estimated partitions coefficients with reported values.

Partition coefficient	This study Expected value (95% Confidence interval)	Nihlén and Johanson (1999)	Kaneko <i>et al.</i> (2000)
Blood/air (P_{ba})	15.6 (7.42 – 32.9)	11.7 ^a	11.6 ± 1.5 ^a
Liver/blood (P_{hb})	1.44 (0.991 – 2.09)	1.68 ^b	2.9 ± 0.3 ^a
Fat/blood (P_{fb})	17.8 (7.28 – 43.6)	12.3 ^b	11.7 ± 2.6 ^a
Rapidly perfused tissue/blood (P_{rb})	1.43 (0.952 – 2.15)	2.34 ^b	1.7 ± 0.4 ^a
Slowly perfused tissue/blood (P_{sb})	1.53 (1.05 – 2.24)	1.71 ^b	1.9 ± 0.5 ^a

^a measured value,^b predicted value

absolute value of the ratio of 0.5 or higher was considered highly sensitive, 0.2–0.5 was considered moderately sensitive, and less than 0.2 was considered less sensitive.

We evaluated the reliability of the dose metrics estimated by the PBK model to be high, moderate, or low based on the results of the uncertainty and sensitivity analyses, referring to WHO guidelines (2010).

RESULTS AND DISCUSSION

Partition coefficients and metabolic parameters of ETBE in humans

Chemicals are classified into four categories based on the dominant intermolecular interactions: 1) van der Waals forces, 2) van der Waals forces and dipole–dipole interactions, 3) van der Waals forces, dipole–dipole interactions, and hydrogen bonds, and 4) ion–ion interactions. Overall, the dipole, hydrogen bond, and ionic interactions are one, two, and three orders of magnitude larger than the van der Waals force, respectively. Because ETBE belongs to a category in which the van der Waals force and dipole–dipole interaction play significant roles, the estimated equations were derived by linear regression of the previously reported values of the partition coefficients and physical properties (Henry's law constant or log D) of chemicals in this category. Table S2 and Fig. S1 of Supplementary material 2 show the results of the linear regression between the common logarithmic values of the blood/air partition coefficients of oxygen-containing compounds in humans and the common logarithmic values of their Henry's law constants. The adjusted R-squared was 0.88, and the *p*-values for the regression intercept and slope were both less than 0.01. Furthermore, Table S4 and Fig. S2 of Supplementary material 2 show the result of the linear regression between the common logarithm of

the liver/blood partition coefficient and log D. The adjusted R-squared was 0.65, and the regression equation's intercept and slope *p*-values were both less than 0.05. Similarly, the results of the linear regression between the common logarithmic values of the partition coefficients and log D for fat/blood, richly perfused tissue/blood, and slowly perfused tissue/blood are shown in Table S6 and Fig. S3, Table S8, and Fig. S4, and Table S10 and Fig. S5 of Supplementary material 2, respectively. Except for the intercepts for the fat/blood partition coefficient, the intercepts and slopes of the regression equations for these tissue/blood partition coefficients also had *p* values of less than 0.05.

The values of Henry's law constant (166 Pa·m³/mol) and log D (1.479) of ETBE are both within the scope of each linear regression equation. According to the procedure described in the "Partition coefficients and metabolic parameters" section, the statistics of the probability density functions of the blood/air and tissue/blood partition coefficients of ETBE were calculated, as shown in Table 2. The expected values of blood/air, liver/blood, fat/blood, richly perfused tissue/blood, and slowly perfused tissue/blood partition coefficients were 15.6, 1.44, 17.8, 1.43, and 1.53, respectively. As shown in Table 3, these values are 0.61 to 1.5 times the human measurements or estimates reported by Nihlén and Johanson (1999), and except for the rapidly perfused tissue/blood partition coefficient, their reported values were within the 95% confidence interval of each partition coefficient estimated in this study. Furthermore, except for the liver/blood partition coefficient, no significant differences were discovered between the rat partition coefficients measured by Kaneko *et al.* (2000) and our corresponding estimates.

Tables S3 and S6 of the Supplementary material 2 summarize the contributions (CRi) and statistics of each

structural fragment to $\log V_{\max}$ and $\log K_m$ obtained from Free–Wilson analysis, respectively. Adjusted R-squared values were 0.80 and 0.56, respectively. Also, both p -values are less than 0.01, indicating that significant relationships exist between response variables ($\log V_{\max}$ and $\log K_m$) and explanatory variables (structural fragments).

According to the procedure described in the “Partition coefficients and metabolic parameters” section, the statistics of the probability density functions of $V_{\max}$ and K_m of ETBE were also calculated from the contribution of structural fragments and the frequency of occurrence of structural fragments in ETBE, as shown in Table 2. The expected values for $V_{\max}$ and K_m for ETBE were 1.11 mg/hr/kg (29.6 $\mu\text{mol/hr/kg}$) and 0.09 mg/L (10.7 μM), respectively. The results had a large logarithmic standard deviation and were significantly lower than $V_{\max}$ (499 $\mu\text{mol/hr/kg}$) and K_m (1248 μM) used in the rat PBK model of Borghoff *et al.* (2017).

The Free–Wilson method using structural fragments seemed to apply to the estimation of metabolic parameters of $V_{\max}$ and K_m . However, the number of reported value data for $V_{\max}$ and K_m was insufficient to be analyzed by category, such as partition coefficients, moreover, significant increases in these data were not expected. Metabolism-related parameters are difficult to predict using *in silico* methods; nonetheless, hepatic clearance of many chemicals was measured in *in vitro* studies using liver slices and cell fractions recently (Madden *et al.*, 2019). Even in these cases, PBK modeling coupled with the Monte Carlo simulation demonstrated in this study can be applied to the uncertainty analysis of toxicokinetics related to measurement error in *in vitro* studies. The validity of our estimated $V_{\max}$ and K_m values will be further discussed in the “Sensitivity and uncertainty of internal dose metrics” section.

PBK model simulation of ETBE

Figure 2 shows the median estimated venous blood ETBE concentrations and their 95% confidence intervals, with the measurements of Nihlén *et al.* (1998). At an inhaled ETBE concentration of 5 ppm, the 95% confidence interval for venous blood concentrations was 0.60–1.8 times the median during the exposure, but after the end of exposure, the median and 95% confidence interval deviated as the blood concentration decreased, and the 95% confidence interval for 240 minutes at the end of exposure was 0.25–5.4 times the median. In the case of 25 ppm exposure, the 95% confidence intervals during and after exposure were 0.58–1.7 and 0.23–5.0 times the median, respectively. Furthermore, the 95% confidence intervals during and after 50 ppm exposure were 0.56–

Fig. 2. Estimation of venous blood concentrations of ETBE during and after inhalation exposure at three concentrations using the PBK model along with Monte Carlo simulation. (A) 5 ppm, (B) 25 ppm, (C) 50 ppm inhalation, 120 min at 50 W light exercise. Lines: predicted ETBE venous blood concentrations (μM). The orange area represents the 95% confidence interval. Points: data from Nihlén *et al.* (1998).

1.7 and 0.22–4.7 times the median, respectively. However, the 95% confidence intervals for blood concentration AUC were 0.54–2.4 (5 ppm), 0.48–2.1 (25 ppm), and 0.47–2.1 (50 ppm) times the median even 4 hr at the end of the exposure. The 95% confidence intervals for AUC immediately after the end of the exposure were 0.65–1.6 (5 ppm), 0.62–1.5 (25 ppm), and 0.61–1.6 (50 ppm) times the median, indicating that the differences between the median and 95% confidence intervals for blood concentrations at the end of the exposure had minimal effect on AUC. Although the values measured by Nihlén *et al.* (1998) were within the 95% confidence interval for all exposure concentrations, blood concentrations during and after exposure tend to be overestimated overall. At 5 ppm exposure, the median-estimated concentration was 1.15 times the respective measured values (mean, range: 0.51–1.6 times), at 25 ppm exposure, the median-estimated concentration was 1.6 times the respective measured values (mean, range: 0.55–2.4 times) and at 50 ppm exposure, the median-estimated concentration was 1.8 times the respective measured values (mean, range: 0.51–2.7 times). The estimates tend to be overestimated, especially for post-exposure concentrations. Nonetheless, at the ETBE concentrations of 5, 25 and 50 ppm, the medians of calculated $C_{\max}$ were 1.39, 7.18, and 14.6 μM , respectively, and the medians of calculated AUC until 4 hr after the end of exposure were 249, 1290, and 2700 $\mu\text{M}\cdot\text{min}$, respectively. These almost linear increases of $C_{\max}$ and AUC values, which are 10.5-fold and 10.8-fold increases from 5 to 50 ppm, respectively, suggest the absence of saturation of ETBE metabolism at these concentrations and consistency with the human ETBE kinetics (Nihlén *et al.*, 1998). Post-exposure blood concentrations displayed a two-phase disappearance, with half-lives of 20 min and 1.7 hr for 5 ppm exposure, 23 min and 1.8 hr for 25 ppm exposure, and 24 min and 1.7 hr for 50 ppm exposure. Nihlén *et al.* (1998) reported four phases of ETBE disappearance in blood, with half-lives of 1.8 min, 20 min, 2.1 hr, and 33 hr at 5 ppm exposure, 1.2 min, 15 min, 1.5 hr, and 24 hr at 25 ppm exposure and 2 min, 1.9 min, 1.5 hr, and 27 hr at 50 ppm exposure. The half-lives of phase 1 and phase 2 obtained from our PBK model calculations were considered to correspond to their phase 2 and phase 3 half-lives, and the half-lives were almost the same, respectively.

Sensitivity and uncertainty of internal dose metrics

A quantitative evaluation of sensitivity and uncertainty of parameter estimates on the concentration-time profiles and the internal exposure indices can help identify parameters that require further refinement. Figure 3 shows the

A

		UNCERTAINTY		
		High	Medium	Low
SENSITIVITY	High			P_{ba}
	Medium			
	Low			$V_{\max}, K_m, P_{fb}, P_{rb}, P_{sb}, P_{lb}, P_{gb}, P_b$

B

		UNCERTAINTY		
		High	Medium	Low
SENSITIVITY	High		P_{ba}	
	Medium		$V_{\max}$	
	Low		K_m	$P_{fb}, P_{rb}, P_{sb}, P_{lb}, P_{gb}, P_b$

Fig. 3. Results of uncertainty and sensitivity analysis on (A) $C_{\max}$ and (B) AUC in human PBK model simulations of ETBE. Based on the matrix of uncertainty indices and sensitivity ratios, the reliability of the PBK model predictions was defined as moderate when at least the uncertainty and sensitivity were moderate, and as high when both uncertainty and sensitivity were low.

results of the sensitivity and uncertainty analyses of the internal dose metrics ($C_{\max}$ and AUC) of 50-ppm ETBE inhalation exposure. The sensitivity ratio of $C_{\max}$ to the blood/air partition coefficient was 0.50, indicating high sensitivity, while the sensitivity ratios of $C_{\max}$ to other partition coefficients and metabolic parameters were less than 0.2, indicating low sensitivity. Furthermore, the $C_{\max}$ uncertainty indices related to all partition coefficients and metabolic parameters were less than 0.3, indicating low uncertainty. Conversely, AUC sensitivity was high (sensitivity ratio: 0.58) to the blood/air partition coefficient, moderate (sensitivity ratio: 0.27) to $V_{\max}$, and low (sensitivity ratio: < 0.20) to other partition coefficients and K_m . Furthermore, the AUC uncertainty indices were 0.35, 0.47, and 0.46 for the blood/air partition coefficient, $V_{\max}$, and K_m , respectively, and less than 0.3 for other partition coefficients, indicating that the uncertainties for AUC of blood/air partition coefficient, $V_{\max}$, and K_m were moderate.

Blood ETBE concentrations were determined to be highly sensitive to the blood/air partition coefficient (P_{ba}), indicating that air/blood partitioning in the alveoli is a crucial factor in determining uptake into the body

via inhalation exposure to volatile organic chemicals. The estimated P_{ba} value in this study was 1.3 times higher than the measured value (Nihlén and Johanson, 1999), and this difference may be one of the factors that resulted in estimated blood concentrations that were approximately 2 times higher than the measured value. The blood concentrations had low sensitivity for the other partition coefficients and both V_{max} and K_m , and the uncertainty in the blood concentration estimates due to the uncertainty in the partition coefficients and metabolic parameters estimates was also low. However, the results of the evaluation of sensitivity and uncertainty on AUC indicated that the uncertainty of AUC estimates due to uncertainty in P_{ba} , V_{max} , and K_m was moderate and sensitivity was high, moderate, and low for P_{ba} , V_{max} , and K_m , respectively. Overall, the reliability of C_{max} and AUC that was estimated using parameter values complemented by the *in silico* methods were judged to be high and moderate, respectively.

The uncertainties of the V_{max} and K_m values (shown as logarithmic standard deviations) were one order of magnitude larger than those of the partition coefficients (Table 2). These uncertainties directly unaffected blood concentrations such as C_{max} , but affect AUC as an indicator of cumulative internal exposures. In cases of volatile organic chemicals like ETBE, absorption and excretion of chemicals via the lungs greatly contribute to changes of the blood concentrations. Therefore, the greater uncertainties of V_{max} and K_m were not so impactful for this substance. The degree of uncertainty and sensitivity will depend on the physicochemical and biochemical properties of the chemical and the parameter estimation method used. Therefore, when supplementing *in silico* methods, it is critical to conduct quantitative analyses of uncertainty and sensitivity using Monte Carlo simulation to confirm that the PBK model is reliable enough for the intended purpose.

In conclusion, the partition coefficients and metabolic characteristics calculated by *in silico* methods were used to develop a PBK model of ETBE after inhalation exposure in humans. The blood concentration-time profiles and ETBE half-lives in the model did not differ significantly from those measured in humans, and the reliability of the model was demonstrated with the uncertainty and sensitivity analyses of C_{max} and AUC by Monte Carlo simulation. These results imply that *in silico* estimation complements the missing parameters in the model and that the reliability of the toxicokinetics estimated from those complementary parameters can be assessed using Monte Carlo simulation. To make this technique properly apply to many chemicals in the absence of TK data, further investigations are necessary for other types of chemicals, QSPR estimation methods, and other exposure

routes than inhalation.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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