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Estimating metabolic rate for butadiene at steady state using a Bayesian physiologically-based pharmacokinetic model

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Abstract In a study of 133 volunteer subjects, demographic, physiologic and pharmacokinetic data through exposure to 1,3-Butadiene (BD) were collected in order to estimate the percentage of BD concentration metabolized at steady state, and to determine whether this percentage varies across gender, racial, and age groups. During the 20 min of continuous exposure to 2 parts per million (ppm) of BD, five measurements of exhaled concentration were made on each subject. In the following 40 min washout period, another five measurements were collected. A Bayesian hierarchical compartmental physiologically-based pharmacokinetic model (PKPB) was used. Using prior information on the model parameters, Markov Chain Monte Carlo (MCMC) simulation was conducted to obtain posterior distributions. The overall estimate of the mean percent of BD metabolized at steady state was 12.7% (95% credible interval: 7.7–17.8%). There was no significant difference in gender with males having a mean of 13.5%, and females 12.3%. Among the racial groups, Hispanic (13.9%), White (13.0%), Asian (12.1%), and Black (10.9%), the significant difference came from the difference between Black and Hispanic with a 95% credible interval from −5.63 to −0.30%. Those older than 30 years had a mean of 12.2% versus 12.9% for the

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younger group; although this was not a statistically significant difference. Given a constant inhalation input of 2 ppm, at steady state, the overall mean exhaled concentration was estimated to be 1.75 ppm (95% credible interval: 1.64–1.84). An equivalent parameter, first-order metabolic rate constant, was also estimated and found to be consistent with the percent of BD metabolized at steady state across gender, race, and age strata.

Keywords Metabolic rate constant · Steady state · Bayesian hierarchical model · Compartmental model · Markov Chain Monte Carlo · Differential equation · Nonlinear model · Random effects

1 Introduction

1,3-Butadiene (BD), a known human carcinogen, is a by-product of petroleum processing, and is usually found in synthetic rubber and plastic products. Exposure to BD is usually through breathing in contaminated air, consuming food stored in rubber containers, or having skin contact with gasoline (ATSDR 1993). Acute high-level exposure (>1,000 ppm) to BD can cause a number of adverse effects such as nausea, fatigue, poor vision, lower blood pressure, and other symptoms of neurological effects. BD has also been classified by the United States department of health and human services as potentially having properties and adverse health effects of a carcinogen. Spills of more than 1 pound of this chemical into the environment must be reported to the environmental protection agency (ATSDR 1993). OSHA (the occupational safety and health administration) has set the allowable occupational exposure limit of BD to a time-weighted average of 1 ppm for an 8-h work shift, and an average of 5 ppm for a 15 min exposure. There are standard methods to measure BD exposure in an individual developed by NIOSH (National institute for occupational safety and health), Method 1024, and OSHA, Method 56.

A number of animal studies using rats and mice have been conducted to evaluate the toxicity and carcinogenicity of BD exposure. Seaton et al. (1996) and Himmelstein et al. (1996) suggested that differences in metabolic activation of BD may lead to species differences in carcinogenicity sensitivity. Thornton-Manning et al. (1996) reported gender differences observed in BD metabolism of Sprague–Dawley rats. Himmelstein et al. (1996) identified male mice's lung and liver to be among the tissues that developed tumors at or above 62.5 ppm of BD exposure; whereas, females exposed to as low as only 6 ppm also developed tumors. In animal studies of mice and rats, pharmacokinetics models have also been used to estimate the rate of BD metabolism (Csanady et al. 1996; Himmelstein et al. 1996; Leavens and Bond 1996; Shargel and Yu 1999; Sweeney et al. 1996). The large differences in animal metabolic rates have led to considerable uncertainty in the estimates of human metabolism of BD. Melnick and Huff (1993) and Lang (1994) found that rats require larger doses than mice to induce cancer. They identified the lowest level of butadiene concentration (6.25 ppm) that could induce cancer in mice. Other researchers reported higher levels of cancer-induced

butadiene exposure of 1,000 to 8,000 ppm for rats, and 624 to 1,250 ppm for mice. Differences of butadiene metabolites such as monoepoxide have also been shown to exist between monkeys, mice, and rats (Kohn and Melnick 1993, 2000). Monkeys had lower level of butadiene monoepoxide than either rats or mice. Thus making inferences to humans using observations, and model results from mice or rat studies needs to be done with caution. Factors such as rate of accumulation of metabolites, formation of DNA adducts, and efficiency of DNA repair can play a crucial role in the differences in the butadiene metabolism process between primates and rodents (Kohn and Melnick 1993; Lang 1994; Melnick and Huff 1993).

Pharmacokinetic models are frequently used to help understand and model the interaction of internal processes of storage, distribution, metabolism, and excretion of the chemical compound from the body through time. Model parameters such as metabolic rate can be estimated. Physiologically-based pharmacokinetic (PBPK) models have been developed and used (Filser et al. 1993; Kohn and Melnick 2000, 2001) to estimate butadiene metabolism rates, distribution and clearance of metabolite concentrations in mice, rats, and humans. However, these authors have only used non-Bayesian estimation methods such as iteratively reweighted least squares via software such as SCoPfit. They have also indicated identifiability as a common estimation problem in these models. The use of known prior information on the model parameters was not taken into account in these models. The traditional frequentist approach of using a mixture of exponential functions, or simple one or two-compartmental models results in a host of potential problems including statistical issues of identifiability, convergence, as well as the possibility of unrealistic physiologic models (Gelman et al. 1996).

In this paper, we demonstrated an advantageous approach using Bayesian analysis (Gelman et al. 1996) for the estimation of the posterior distribution of human parameters in a physiologically-based pharmacokinetic (PBPK) model that was extrapolated to steady state conditions, which allowed ease of interpretation. The Bayesian approach also made use of available prior information for the estimation of the posterior distribution of the model parameters. The steady state analysis was based on a general three-compartmental PBPK model which made use of the observed physiologic data such as height, weight, age, gender, BD blood solubility, and breathing activity during the tests. Prior information on model parameters were also incorporated into the statistical estimation process to obtain the person-specific posterior estimates for the posterior distribution of the model parameters. Based on the characteristics of our experimental exposure and the PBPK model, if the subjects had been exposed for a very long time (unethical and experimentally impractical), they would reach a steady-state where the amount of metabolism per minute would be equal to the difference between the amount of BD inhaled per minute minus the amount exhaled. Although this could not be done in practice, it can be simulated in the Bayesian context given the observed breath data, the posterior estimates of the person-specific parameters, the constant inhaled BD amount, and the time at which the amount of exhaled concentration reached steady state. The result was an easily interpretable estimate of the metabolic rate.

2 Materials and method

2.1 Data description

As described in more detail previously (Lin et al. 2001, 2002), our study involved a group of 144 volunteers. Human subjects committee of the Harvard school of public health approved the study. Data from 11 persons were dropped as unsuitable for analysis due to problems with missing data or unrealistic data values (8 due to instrument malfunction, 2 without blood data, and 1 with outlier values), yielding the remaining 133 subjects (71 males and 62 females) for analysis.

In Table 1, we reported the summary data for gender, ethnicity, physiologic and BD chemical solubility characteristics. Thirty two percent were Asian, 13% Black, 20% Hispanic, and 29% Caucasian, with seven subjects in "other race" groups (near Eastern and Indian subcontinent). The sample had an average age of 29.7 ± 8.2. Age distribution was skewed to the right due to six subjects whose age ranged from 52 to 62. Male subjects were significantly taller by 0.13 m (p-value = 0.0001), and about 15 kilograms heavier than females (p-value = 0.0001). Hispanic and Asian subjects were younger, having an average age of 28 versus about 32 for Black and Caucasian subjects (p-value = 0.025). Body mass index (BMI) was derived from the measured variables (weight/height^2) to have a mean of 24.7 ± 4.9 kg/m^2. Caucasian subjects had larger BMI mean compared to those of the non-caucasian subjects (p-value = 0.003).

Table 1 Demographic, physiologic and BD chemical solubility characteristics ($n = 133$)

Sex (%)	
Male	53
Female	47
Ethnicity (%)	
Caucasian	29
Black	13
Asian	32
Hispanic	20
Other	4
Physiologic characteristics (mean ± SD)	
Age (years)	29.7 ± 8.2
Height (m)	1.68 ± 0.1
Weight (kg)	70.5 ± 17.5
Body mass index (kg/m^2)	24.7 ± 4.9
Number of breaths taken per minute	13.4 ± 3.0
Minute ventilation rate (L/min)	6.9 ± 1.6
Tidal volume (L)	5.3 ± 1.5
Dead space volume per minute (L/m)	1.5 ± 0.5
Chemical solubility characteristics of BD	
Blood/air partition coefficient	1.5 ± 0.3

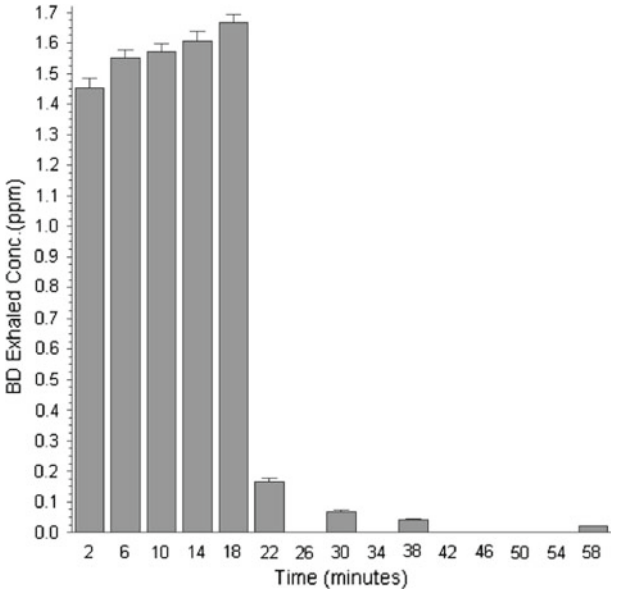

Fig. 1 Mean and standard deviation of measured exhaled concentration of BD

Subjects were continuously exposed to 2 ppm of BD in the laboratory for 20 min. Five measurements of breath samples were taken during the 20 min exposure at the 2nd, 5th, 10th, 15th, and 19th min; and another five measurements were taken during the 40 min pure-air washout period (at the 21st, 22nd, 28th, 38th, and 58th min). Figure 1 shows the distribution of exhaled concentration at each of the ten time points. Urine and blood samples were also taken. Variable number of breaths taken per minute varied from 8 to 22 with a mean of 13.4 ± 3.0 breaths. This variable was used to check the calibration of the breathing monitor, and also to derive the alveolar ventilation rate. Variable minute ventilation rate (L/min) represented the total volume of gas entering the lung per minute. This total volume per minute was equal to the alveolar ventilation rate, and the dead space ventilation rate. The total dead space volume per minute was computed by multiplying the number of breaths per minute and the dead space volume of gas per breath. minute ventilation rate varied from 2.9 to 13.2 L/min, with a mean of 6.9 ± 1.6. The dead space volume per minute was defined as the total volume of gas inhaled but not used in gas exchange. The regions that contained the dead space volume were inside the face mask, the mouth, the trachea, and areas in the alveoli where there was no gas exchange due to the lack of blood flowing through. Dead space volume per minute varied from 0.65 to 2.82 L/min with a mean of 1.5 ± 0.5 L/min. Tidal volume, the total amount of air breathed in or out, ranged from 2.7 to 10.8 L, with a mean of 5.3 ± 1.5. The variable blood/air partition coefficient, used to assess the solubility of BD in blood, was defined as the equilibrium ratio of the blood to air concentrations, and ranged from 0.7 to 2.6, with a mean of 1.5 ± 0.3. In the statistical analysis section below, we have explained how these variables would be used in the model estimation process.

The individual-level demographic and physiologic variables listed in Table 1 were used as input variables in the PBPK model to estimate exhaled BD concentration, and the percent of BD metabolized at steady state.

2.2 Statistical analysis

We will first explain the PBPK model, then the likelihood function, the priors, and the estimation procedure for the posterior function. At each step, we will give the definition of the variables involved in these functions, and whether they are fixed or random variables, measured at the individual or population (constant) level.

2.2.1 The PBPK model

First, we set up a PBPK model based on the assumption that tissues could be grouped together into three compartments. In our previous work, we considered also a four-compartment model, and provided an explanation as to why we thought the three-compartment model fitted better (Mezzetti et al. 2003). Figure 2 shows a heuristic overview of the model. BD was inhaled, then distributed by the blood flow to the compartments. BD was then carried back to the lung by blood flow to be exhaled. First-order metabolic rate was assumed to exist only in the well-perfused tissue compartment.

The time-dependent concentration of BD for each subject in each of the three compartments was estimated using the first-order differential equations ("Appendix", Eqs. 1 to 3), where $Q_{pp}(i,t)$, $Q_{fat}(i,t)$, $Q_{wp}(i,t)$ were the quantities of BD in poorly perfused, well-perfused, and fat tissue compartment, respectively, at a particular time point t for person i. The quantity on the left-hand side of each of these three equation denoted the first derivative of BD concentration evaluated at time t with respect with time t. It was the instantaneous rate of change of BD concentration in the particular tissue compartment. On the right-hand side of each of the three differential equations above, $F_{pp}(i)$, $F_{wp}(i)$, $F_{fat}(i)$ represented the volume of blood flow to the tissue compartment, and were computed as

$$F_{pp}(i) = percent\_flow_{pp} * flow\_total(i), F_{fat}(i) = percent\_flow_{fat} * flow\_total(i)$$
$$F_{wp}(i) = (1 - percent\_flow_{fat} - percent\_flow_{pp}) * flow\_total(i) \text{ where}$$
$$flow\_total(i) = (flow\_pulmonary(i) - number\_breaths(i) * deadspace(i)/1000)/1.14.$$

The percentage of blood flow $percent\_flow_{pp}$, $percent\_flow_{fat}$, $percent\_flow_{wp}$ were summed to 100% with $percent\_flow_{wp}$ treated as population constant for all subjects. $percent\_flow_{pp}$ and $percent\_flow_{fat}$ were treated as random. The variables $flow\_pulmonary$, $number\_breaths$, and $deadspace$ were measured once for each subject with the descriptive summary of their distributions reported in Table 1. $V_{pp}(i)$, $V_{wp}(i)$, $V_{fat}(i)$ were effective volumes of the tissue compartments, defined as a product of the percentage of body weight due to fat, poorly-perfused,

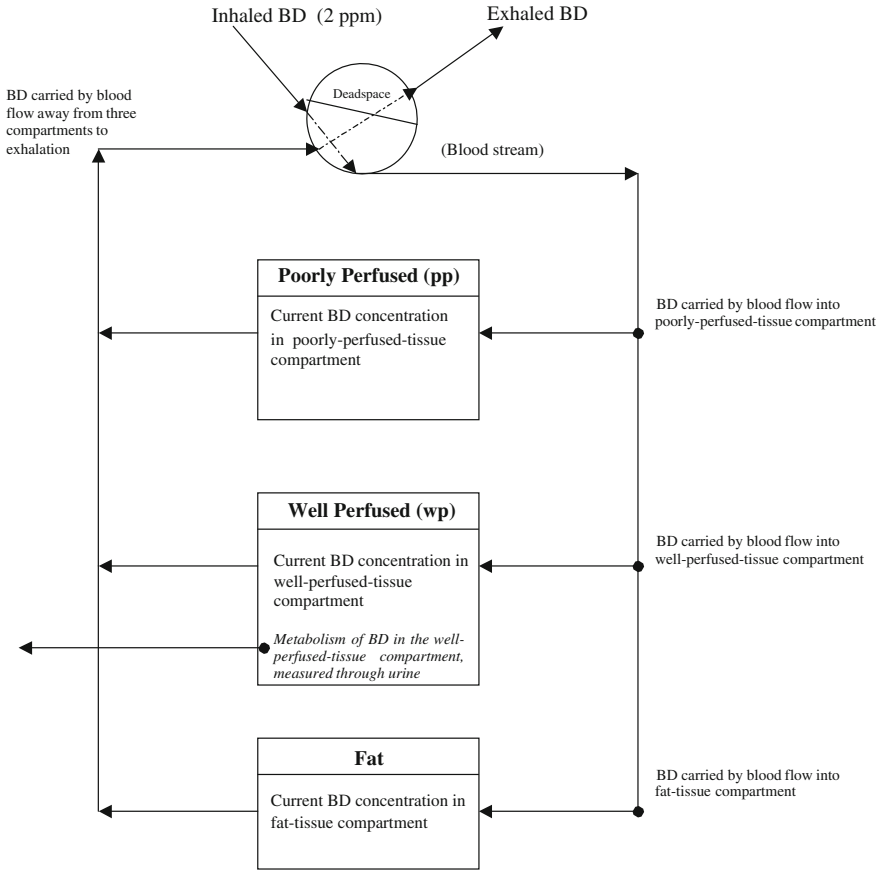

Fig. 2 Three-compartmental PBPK model for BD

or well-perfused tissue ($percent\_weight_{wp}$) and the total body weight which was measured for each subject. $percent\_weight_{wp}$ was random. The percentage of body weight due to the particular tissue type was computed as a function of body mass index, gender, and age, which were measured for each subject. PC_{pp}, PC_{wp}, PC_{fat} were partition coefficients. PC_{wp}, PC_{fat} were defined as population constants. PC_{pp} was assumed to be random. The reason for PC_{pp} to be random was because in our previous work (Mezzetti et al. 2003), we found PC_{pp} to be associated with individual-level demographics of age, sex, and ethnicity. Since subjects were random, we treated PC_{pp} as random. These partition coefficients represented the ratio at equilibrium of the concentration of BD in the blood to the concentration of BD in the corresponding tissue compartment. k_{met} represented the first-order rate of metabolism, and was to be estimated.

The arterial blood BD concentration C_{art} was calculated as in Eq. 4 ("Appendix"), where f_{ds} was the percentage of dead space computed for each individual, PC_{ab} was the blood–air partition coefficient, computed as the steady-state ratio of BD

concentration in venous blood to the BD concentration in headspace air (Lin et al. 2001). $C_{inhaled}$ was the amount of BD given to each subject (continuous exposure of 2 ppm of BD for 20 min). In Eqs. 1 to 3, the first quantity represented the amount of BD carried to the tissue compartment by the blood flow, and the second quantity represented the amount of BD leaving the tissue compartment. The difference between these two quantities yielded the time-specific change in the amount of BD in the compartment, or the rate of change. For the well-perfused tissue compartment, an additional assumption was made that metabolism occurred here so that the amount leaving the well-perfused tissue compartment was due to both the blood flow, and the metabolic process (first-order rate k_{met}). The concentration of exhaled BD was the sum of the fraction of inhaled concentration in the airway dead space and in the alveolar airway area. The exhaled concentration $C_{exhaled}$ was expressed as in Eq. 5 ("Appendix").

2.2.2 The likelihood function

Next, for the estimation process, we first set up the posterior distribution which was a product of the likelihood function and the prior distributions. Each of the 133 subjects had data for $C_{exhaled}$, $flow\_pulmonary$, f_{ds}, and PC_{ab} in addition to weight, height, gender, age, the number of breaths taken, and the mentioned fixed population constants ($percent\_flow_{wp}$, PC_{wp}, PC_{fat}) and random variables PC_{pp}, $percent\_flow_{pp}$, $percent\_weight_{wp}$, k_{met}, $percent\_flow_{fat}$ as input in the model. F_{pp}, F_{wp}, F_{fat} and V_{pp}, V_{wp}, V_{fat} were computed quantities from these input quantities. We formulated the likelihood function ("Appendix", Eq. 6) using the joint distribution of $C_{exhaled}$, $flow\_pulmonary$, f_{ds}, and PC_{ab}, each one assumed to have a log-normal density function.

2.2.3 MCMC for estimation of the posterior distributions

In Eqs. 1 to 5, we were interested in obtaining the estimates of the posterior distributions for eight random parameters (PC_{pp}, $percent\_flow_{pp}$, $percent\_weight_{wp}$, k_{met}, $percent\_flow_{fat}$, $flow\_pulmonary$, f_{ds}, and PC_{ab}) for each of the 133 subjects since we also assumed subjects to be random. Obtaining *a priori* information for these parameters was a challenge. We used reference values derived mainly from the Caucasian population. We used Markov Chain Monte Carlo (MCMC) approach to draw samples of these parameters from the posterior distribution (Gelman et al. 1996). Each parameter (such as the 133 PC_{ab}'s) came from a population distribution with given mean, and variance. These eight population means and eight population variances were assigned prior information. Tables 2 and 3 show the prior information available or assumed for the population means and variances. The distribution of the population mean parameter was assumed to follow a truncated log normal distribution with mean, variance, minimum, and maximum which were shown on the natural scale. The population variances of the parameters were assumed to follow the inverse gamma distribution with the shape parameter of 1. The posterior distribution of the parameters was formed from the product of the distributions of the population parameters, the likelihood function (Eq. 6), and the given prior distributions. There was a total of 1,064

Table 2 Prior information for population mean

Parameter[a]	Distribution	Mean	Variance	Minimum	Maximum
PC_{pp}	Truncated log normal	1.80	5.00	0.20	4.00
$percent\_flow_{pp}$	Truncated log normal	0.35	5.00	0.01	0.40
$percent\_weight_{wp}$	Truncated log normal	0.20	5.00	0.01	0.40
k_{met}	Uniform	0.25		0.01	0.50
$percent\_flow_{fat}$	Truncated log normal	0.10	5.00	0.03	0.18
$flow\_pulmonary$	Truncated log normal	5.00	5.00	3.73	13.40
f_{ds}	Truncated log normal	0.70	5.00	0.20	0.95
PC_{ab}	Truncated log normal	2.00	5.00	0.20	5.00

[a] Parameters of the log normal distribution are given on the natural scale (i.e. mean and variance are geometric mean and variance)

Table 3 Prior information for population variance

Parameter	Distribution	Shape	Scale
PC_{pp}	Inverse gamma	1	0.200
$percent\_flow_{pp}$	Inverse gamma	1	0.200
$percent\_weight_{wp}$	Inverse gamma	1	0.200
k_{met}	Inverse gamma	1	0.693
$percent\_flow_{fat}$	Inverse gamma	1	0.200
$flow\_pulmonary$	Inverse gamma	1	0.262
f_{ds}	Inverse gamma	1	0.182
PC_{ab}	Inverse gamma	1	0.182

(133 × 8) parameters at the individual-specific level (not counting the measurement error variance), 8 mean parameters, and 8 variance parameters for the population level. Thus the total number of parameters to be estimated was 1,080. Differential equations and Markov Chain Monte Carlo simulation (Metropolis-Hastings algorithm) were carried out using the software MCSim (Bois and Maszle 1997; Bois et al. 1999) to obtain sample distributions of the parameters from their joint posterior distribution. The first 10,000 observations were used as a burn-in period and every fifth observation from the last 5,000 iterations was used to estimate the distribution of the parameters.

2.2.4 Estimation of exhaled concentration at steady state

From the simulated sample of the model parameter values, the steady-state exhaled concentration was calculated by predicting the concentrations at up to 16 days. The long-term exposure was simulated by first obtaining the estimates of the posterior distribution of the parameters from Eqs. 1 to 4. Then inputting the inhaled concentration constant at 2 ppm and duration of inhalation (time in minutes) in Eq. 5, the estimated exhaled concentration was obtained. By inhaled concentration, we meant the 2 ppm

of BD amount inhaled by each subject who was assumed to be in the inhalation chamber and received a constant amount of 2 ppm until steady state was reached. We then examined the estimates of the exhaled concentration at 8, 10, 12, 14, and 16 days. Steady-state concentration was assumed to be reached if the difference between the two consecutive estimates of exhaled concentrations was less than 0.00001. All subjects satisfied this criterion at or before 16 days.

2.2.5 Estimation and comparison of percent BD metabolized at steady state

Based on the steady-state concentration, the percent of metabolized BD was calculated from the input continuous inhalation amount of 2 ppm. Trace plots of the values of parameter by the iteration number were examined to observe the convergence characteristic of each parameter for each subject. The mean, 2.5, and 97.5th percentile of the each subject's parameter distribution were obtained and averaged to provide the 95% credible interval. For the comparison between the sexes, the data were first stratified by sex, then MCMC simulation was done separately for each sex-specific group. The same procedure was also done for each of the ethnic groups, and age groups. Posterior mean and 95% credible intervals were calculated and reported. We were interested in testing for the difference in the population means of percent of metabolized BD, for instance, between male and female subjects. The frequentist approach would be to use a two-sample t-test, a linear model, or a non-parametric Wilcoxon test to test the hypothesis of the means being equal. However, we had 1,000 simulated values for this parameter (percent of metabolized BD) for each of the 71 male subjects, and 62 female subjects, and we were interested in obtaining the 95% credible interval of the difference in percent of metabolized BD between male and female subjects. To compute the 95% credible interval of the difference, we needed to obtain the distribution of the differences. Each of our 1,000 simulated values for this parameter can be treated as an estimate from a random sample. For each sample, we computed the difference between male and female. Thus, we have a distribution of 1,000 differences. We sorted these differences in ascending order and computed the 2.5 and 97.5th percentile. This was the 95% credible interval for the difference in the percent of metabolized BD that we reported. If this interval did not include zero, we concluded that there was a statistically significant difference between the comparison groups. We did similar analysis to compare the race groups, and the groups based on age with the cutoff at 30 years.

3 Results

Metabolism was examined by estimating the exhaled BD concentration at steady state given a subject's model and a simulated steady long -term exposure. The difference between inhaled and exhaled BD at steady state was assumed to have been removed by metabolism. Table 4 shows the overall mean of exhaled BD concentration at steady state to be 1.75 ppm (95% credible interval from 1.64 to 1.84 ppm). The mean percent of BD metabolized at steady-state was 12.7 % (95% CI: 7.7–17.8%). First-order metabolic rate constant K_{met} was estimated to be 0.114/ min (0.053–0.209). Males had

Table 4 Mean and 95% credible interval of posterior estimates

Parameter	All subjects ($n = 113$)	Male ($n = 71$)	Female ($n = 62$)	White ($n = 38$)	Black ($n = 17$)	Hispanic ($n = 26$)	Asian ($n = 45$)	Age $\geq$ 30 ($n = 43$)	Age < 30 ($n = 90$)
K_{met}	0.114 (0.053, 0.209)	0.140 (0.066, 0.248)	0.105 (0.049, 0.197)	0.111 (0.046, 0.234)	0.080[a] (0.036, 0.157)	0.161[a] (0.074, 0.283)	0.108[a] (0.049, 0.203)	0.102 (0.049, 0.190)	0.121 (0.055, 0.217)
Exhale concentration at steady state	1.75 (1.64, 1.84)	1.73 (1.63, 1.83)	1.75 (1.65, 1.85)	1.74 (1.63, 1.84)	1.78 (1.67, 1.88)	1.72 (1.62, 1.83)	1.76 (1.65, 1.86)	1.76 (1.65, 1.85)	1.74 (1.64, 1.84)
Percent of BD metabolized at steady state	12.7 (7.7, 17.8)	13.5 (8.5, 18.6)	12.3 (7.2, 17.6)	13.0 (7.8, 18.6)	10.9[b] (5.7, 16.2)	13.9[b] (8.6, 19.1)	12.1 (6.8, 17.5)	12.2 (7.3, 17.5)	12.9 (7.8, 18.1)

[a] 95% credible interval of the difference in K_{met} between Asian and Hispanic (−9.41%, −1.59%), between Black and Hispanic (−12.21%, −3.55%)
[b] 95% credible interval of the difference in percent of BD metabolized at steady state between Black and Hispanic (−5.63%, −0.30%)

slightly higher mean of percent BD metabolized at steady state than females (13.5% versus 12.3%), though this difference was not statistically significant. Both groups reached a similar level of steady-state concentration. Estimate of K_{met} was much higher for males than females (0.140 versus 0.105) and this was consistent with the higher percentage of metabolized BD in males, 13.5% versus 12.3% for females. Hispanic subjects reached steady-state concentration earliest (1.72 ppm), and Black latest (1.78 ppm). Hispanic group had the largest mean percent of BD metabolized at steady state at 13.9% with a 95% credible interval of 8.6–19.1, while the Black group had the lowest at 10.9% (5.7–16.2). Caucasian group had a mean of 13% (7.8–18.6), and Asian had a mean of 12.1% (6.8–17.5). The mean difference of percent of BD metabolized between Hispanic and Black was significant with 95% credible interval from −5.63 to −0.30%.

The trend of K_{met} for racial group was consistent with the trend of percent BD metabolized at steady state. Hispanic group had the highest K_{met} at 0.161 (0.074–0.283), Caucasian at 0.111 (0.046–0.234), Asian at 0.108 (0.049–0.203), and then Black at 0.080 (0.036–0.157). There was statistically significant difference in K_{met} between Hispanic and Asian (credible interval: −9.41 to −1.59%), and Hispanic and Black (−12.21 to −3.55%). Subjects older than 30 years of age had slightly higher mean percent of BD metabolized (12.9% versus 12.2%, although not significant), and higher K_{met} (0.121 versus 0.102) compared to those 30 or younger.

Figure 3 shows the distribution of the posterior estimates of K_{met} and percent BD metabolized based on all 133,000 estimates (133 subjects, 1,000 estimates each), as well as on 133 individual means.

4 Discussion

We have described an extension of the use of a Bayesian hierarchical physiologically-based pharmacokinetic model to estimate the metabolic rate of BD in human subjects, which was reported earlier by Lin et al. (2002) and Mezzetti et al. (2003). Our question of interest here is the estimation of another quantity (percent BD metabolized at steady-state) that is equivalent to the metabolic rate, and whether this quantity differs between the sexes, race and age groups.

The rationale for estimating the percent of BD metabolized at steady state is that this quantity is easy to interpret and intuitive. Under a constant exposure input, what fraction of the quantity taken in that gets metabolized is a clear and important question. At steady-state, BD enters the body by inhalation and leaves by either metabolism or exhalation. Since the amounts inhaled and exhaled could be directly measured, the amount metabolized (μg per min) could be directly estimated by subtraction. The percent of BD metabolized is defined as the relative difference between the amount of BD exhaled at steady state to the amount of BD inhaled. Expressing the amount of BD metabolized in this manner implies that differences in body size have been normalized, assuming that the amount of air inhaled per unit of body mass per minute is approximately the same for most healthy adults. BD is highly lipophilic, so much will go into the fat before a steady state is achieved. In our model BD can only leave the model by exhalation or metabolism (the first rate limiting step). Urinary metabolites

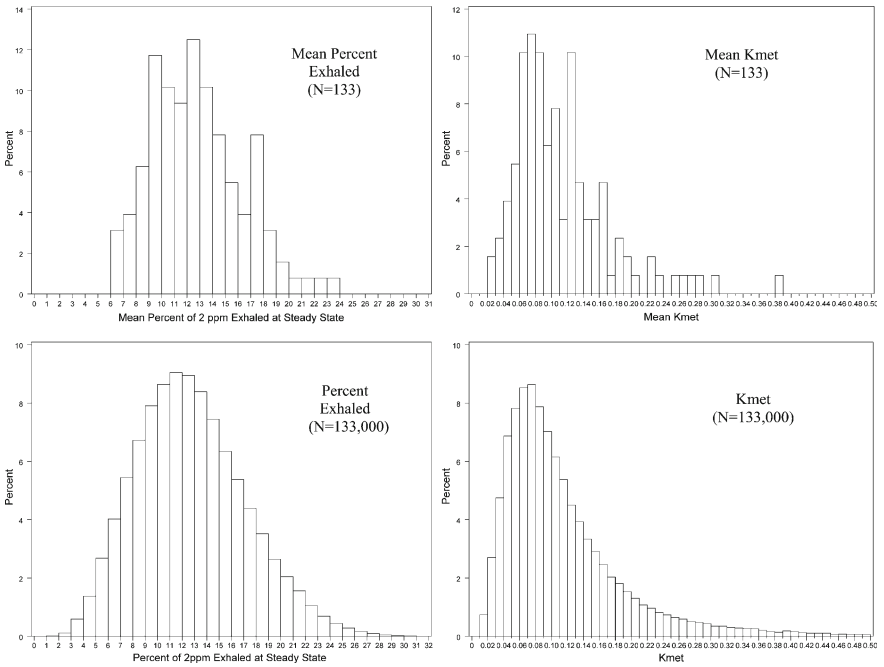

Fig. 3 Distribution of posterior estimates of percent exhaled, and Kmet

are formed, but there is no evidence that a significant amount of the parent compound leaves the system in urine or bile. In the short time frame of the exposure experiment the system certainly does not come to steady state with the body fat. However, in the simulation we extrapolate the internal processes up to 16 days, which showed that the model system had come to steady state, that is no change in the estimated blood or breath levels. Under those extrapolated conditions, the difference between the inhaled and exhaled rates for BD will estimate the amount of metabolized BD.

Whereas the first-order metabolic rate constant K_{met} implies an instantaneous rate of metabolism (per min) within some tissue, which depends on the volume of the metabolizing tissue, BD solubility in the tissue relative to the blood, and the tissue's blood flow. For example, K_{met} has a higher value if all metabolism is assumed to be in the liver, as opposed to metabolism in all vessel-rich tissues. As a result, the percent metabolism and K_{met} are not strictly comparable but are closely related. The patterns of differences across strata for these two parameters are quite consistent (the faster the metabolism by K_{met}, the higher the percentage of BD metabolized).

The finding that males have slightly higher mean metabolic rate than females is not a surprise, since BD is lipid soluble and its uptake is positively correlated with blood/air partition coefficient, and fraction of blood flow to and from fat tissue. Male subjects have higher mean for these two parameters than female subjects, and thus it is expected that male metabolic rate is higher than that of female subjects. Thornton-Manning et al. (1996) carried out animal studies (on rats) and found gender differences as well as species differences (between rats and mice) in BD metabolic rates.

Physiologically-based models have the advantage of making use of animal data to infer about human pharmacokinetics (Shargel and Yu 1999), so the finding of potential sex effect on metabolic rate here seems sensible. Age does not appear to have a large effect on metabolic rate; although, older subjects have slightly higher mean level of fraction ofblood flow to fat tissue, and higher level of air/blood partition coefficient. Lin et al. (2002) also reported that air/blood partition coefficient, a major factor in the uptake of BD was significantly associated with age. However, the younger group (less than 30 years) here had slightly higher K_{met} and percent of BD metabolized at steady state, although the difference was not significant. The difference in the mean levels of metabolic rate among ethnic groups shows Hispanic group to have highest mean percent of BD metabolized, and K_{met} than Caucasian, Asian, and Black. This study confirms that demographic factors such as race, and possibly gender also have an effect on the level of metabolism.

Using a Bayesian hierarchical model and MCMC simulation, we have been able to provide estimates for quantitative differences in metabolic rate and percent metabolism across different groups for these demographic factors. The inclusion of demographic factors and knowledge of the magnitudes of these metabolism estimates can be useful in further study designs of pharmacokinetic and environmental risk assessment studies. Percent metabolism is a more convenient parameter to use in risk estimation because it may be used without PBPK modeling. In our analysis, we have dealt only with butadiene concentration (percent metabolized, and rate of metabolism); however, it is possible that butadiene metabolites such as monoepoxide, diepoxide could indicate stronger heterogeneity among species (Bond et al. 1987). In their analysis, Kohn and Melnick (1993) point out that, in humans but not in mice, the formation of epoxide from butadiene stored in fat tissues would continue after the exposure period is over. It is thus possible that the distribution of butadiene metabolites has larger variability among ethnic, gender, age groups than butadiene concentration itself. The metabolites are possibly better markers for assessing carcinogenicity as well since it is a direct byproduct of butadiene concentration after exposure. The data on metabolites unfortunately are not available to us in this study.

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Appendix

$$\frac{dQ_{pp}(i,t)}{dt} = F_{pp}(i)C_{art}(i,t) - \frac{F_{pp}(i)Q_{pp}(i,t)}{V_{pp}(i)PC_{pp}} \tag{1}$$

$$\frac{dQ_{fat}(i,t)}{dt} = F_{fat}(i)C_{art}(i,t) - \frac{F_{fat}(i)Q_{fat}(i,t)}{V_{fat}(i)PC_{fat}} \tag{2}$$

$$\frac{dQ_{wp}(i,t)}{dt} = F_{wp}(i)C_{art}(i,t) - \frac{F_{wp}(i)Q_{wp}(i,t)}{V_{wp}(i)PC_{wp}} - k_{met}(i)Q_{wp}(i,t) \tag{3}$$

$$C_{art}(i,t) = \frac{flow\_pulmonary(i)(1-f_{ds}(i))C_{inhaled}(i,t) + \frac{F_{pp}(i)Q_{pp}(i)}{V_{pp}(i)PC_{pp}} + \frac{F_{fat}(i)Q_{fat}(i)}{V_{fat}(i)PC_{fat}} + \frac{F_{wp}(i)Q_{wp}(i)}{V_{wp}(i)PC_{wp}}}{flow\_total(i) + flow\_pulmonary(i)(1-f_{ds}(i))/PC_{ab}(i)} \quad (4)$$

$$C_{exhaled}(i,t) = (1-f_{ds}(i))\frac{C_{art}(i,t)}{PC_{ab}(i)} + f_{ds}(i)C_{inhaled}(i,t) \quad (5)$$

$$L = \prod_{i=1}^{133}\left(\prod_{t} LogNormal(C_{exhaled}(i,t))\right) \times LogNormal(flow\_pulmonary(i)) \times LogNormal(f_{ds}(i)) \times LogNormal(PC_{ab}(i)) \quad (6)$$

References

ATSDR (1993) ToxFAQs™ for 1,3-Butadiene. Atlanta, GA: U.S. Department of health and human services, Public Health Service. http://www.atsdr.cdc.gov/tfacts28.html. Accessed 20 Feb 2004

Bois FY, Maszle DR (1997) MCSim: a Monte Carlo simulation program. J Stat Softw 2:1–60

Bois FY, Smith TJ, Gelman AE, Chang HY, Smith AE (1999) Optimal design for a study of butadiene toxicokinetics in humans. Toxicol Sci 49:213–224

Bond JA, Dahl AR, Henderson RF, Irnbaum LS (1987) Species differences in the distribution of inhaled butadiene in tissues. Am Ind Hyg Assoc J 48:867–872

Csanady AG, Kreuzer PE, Baur C, Filser JG (1996) A physiological toxicokinetic model for 1,3-Butadiene in rodents and man: blood concentrations of 1,3- butadiene, its metabolically form epoxides, and of haemoglobin adducts—relevance of glutathione depletion. Toxicology 113:300–305

Filser JG, Johanson G, Kessler W, Kreuzer PE, Stei P, Bauer C, Csanády GA (1993) A pharmacokinetic model to describe toxicokinetic interactions between 1,3-butadiene and styrene in rats: predictions for human exposure. In: Sorsa M, Peltonen K, Vainio H, Hemminki K (eds) Butadiene and Styrene: Assessment of Health Hazards, vol IARC Scientific Publications No. 127, Lyon

Gelman AE, Bois FY, Jiang J (1996) Physiological pharmacokinetics analysis using population modeling and informative prior distributions. J Am Stat Assoc 91:1400–1412

Himmelstein WM, Turner JM, Asgharian B, Bond AJ (1996) Metabolism of 1,3-butadiene: inhalation pharmacokinetics and tissue dosimetry of butadiene epoxides in rats and mice. Toxicology 113:306–309

Kohn MC, Melnick RL (1993) Species differences in the production and clearance of 1,3-butadiene metabolites: a mechanistic model indicates predominantly physiological, not biochemical, control. Carcinogenesis 14:619–628

Kohn MC, Melnick RL (2000) The privileged access model of 1,3-butadiene disposition. Environ Health Perspect 108(5):911–917

Kohn MC, Melnick RL (2001) Physiological modeling of butadiene disposition in mice and rats. Chem Biol Interact 135–136:285–301

Lang L (1994) Baffling butadiene. Environ Health Perspect 102(3):282–285

Leavens LT, Bond AJ (1996) Pharmacokinetic model describing the disposition of butadiene and styrene in mice. Toxicology 113:310–313

Lin YS, Smith TJ, Kelsey KT, Wypij D (2001) Human physiologic factors in respiratory update of 1,3-butadiene. Environ Health Perspect 109:921–926

Lin YS, Smith TJ, Wypij D, Kelsey KT, Sacks MF (2002) Association of blood/air partition coefficient of 1,3-butadiene with blood lipids and albumin. Environ Health Perspect 110(2):165–168

Melnick RL, Huff JE (1993) 1,3-Butadiene induces cancer in experimental animals at all concentrations from 6.25 to 8,000 parts per million. In: Sorsa M, Peltonen K, Vainio H, Hemminki K (eds) Butadiene and styrene: assessment of health hazards. IARC Scientific Publications No. 127, Lyon

Mezzetti M, Ibrahim JG, Bois FY, Ryan LM, Ngo L, Smith TJ (2003) A Bayesian compartmental model for the evaluation of 1,3-Butadiene metabolism. Appl Stat 52(3):291–305

Seaton JM, Plopper GC, Bond AJ (1996) 1,3-Butadiene metabolism by lung airways isolated from mice and rats. Toxicology 113:314–317

Shargel L, Yu A (1999) Applied biopharmaceutics and pharmacokinetics, 4th edn. Appleton and Lange, Stamford, CT, 36–43

Sweeney ML, Himmelstein WM, Schlosser MP, Medinsky AM (1996) Physiologically based pharmacokinetic modeling of blood and tissue epoxide measurements for butadiene. Toxicology 113:318–321

Thornton-Manning RJ, Dahl RA, Bechtold EW, Henderson FR (1996) Gender and species differences in the metabolism of 1,3-butadiene to butadiene monoepoxide and butadiene diepoxide in rodents following low-level inhalation exposures. Toxicology 113:322–325

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