Physiologically Based Pharmacokinetic (PBPK) Modeling of Benzene in Humans: A Bayesian Approach

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Abstract

Benzene is myelotoxic and causes leukemia in humans when they are exposed to high doses (> 1 ppm, more definitely above 10 ppm) for extended periods; however, leukemia risks in humans at lower exposures are uncertain. Benzene occurs widely in the work environment and also in outdoor air, although mostly at concentrations below 1 ppm. It is therefore important to assess the risk to humans when they are exposed to benzene at these low concentrations. In this paper, we describe a physiologically based pharmacokinetic (PBPK) model for the uptake and elimination of benzene in humans to relate the concentration of inhaled and orally administered benzene to the tissue doses of benzene and its key metabolites, benzene oxide, phenol, and hydroquinone. To account for variability among humans, the mathematical model must be integrated into a statistical framework that acknowledges sources of variation in the data due to inherent intra- and inter-individual variation and measurement error and other data collection issues. The main contribution of this work is the estimation of population distributions of key PBPK model parameters. In particular, a Markov Chain Monte Carlo (MCMC) technique is employed to fit the mathematical model to two sets, thereby updating the estimated parameter distributions. We first considered only variability in metabolic parameters, as observed in previous *in-vitro* studies, but found that it was not sufficient to explain observed variability in benzene pharmacokinetics. Variability in physiological parameters, such as organ weights, must also be included to faithfully predict the observed human population variability.

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1. Introduction

Benzene, a toxic industrial solvent, is a component of cigarette smoke and gasoline (Runion and Scott, 1985; Wallace, 1990) and is also widely used in the production of many products. High-level exposure to benzene causes many health problems ranging from dizziness and headaches to anemia and leukemia (Ayres *et al*., 1994). A recent analysis of a prospective cohort study from the Australian petroleum industry showed an increased risk of leukemia; for the highest exposed group (>16 ppm-years) exposure intensity was strongly correlated with leukemia risk, with the increase starting around 0.8-1.6 ppm (Glass *et al*., 2003). These toxic effects likely result from metabolites of benzene formed internally (Ayers *et al*., 1994; Yardley-Jones *et al*., 1991); and hence studying the mechanisms of benzene uptake, metabolism, and elimination through the body can assist in the assessment of acceptable levels of exposure.

Physiologically based pharmacokinetic (PBPK) models are standard tools that are now often used in risk assessment to better extrapolate from experimental animals to humans and from high to low exposures (e.g., Haddad *et al*., 2001; Cahill *et al*., 2003). Cole *et al*. (2001) developed a PBPK model that predicts tissue concentrations of benzene and its key metabolites in mice using metabolic parameters obtained *in vitro*. The PBPK model tissue compartments include the liver, richly perfused and poorly perfused tissues, and adipose tissue. Two additional compartments, the stomach and the alveolar gas-exchange region, were also included to describe oral and inhalation exposures, respectively. This model was later extended to take into account the zonal distribution of enzymes and metabolism in the liver, rather than treating the liver as one homogeneous compartment (Cole, *et al*., 2003). A common characteristic of these PBPK models is that they have single-valued parameters and are deterministic.

However, when the PBPK models are extended to humans, accounting for the multiple sources of variability that will affect dosimetry in humans is important. This hierarchy of variances includes variability among: different studies, individuals within each study, and measurements taken from each individual. To properly account for the variability at any of these levels PBPK models should be integrated into a statistical framework that acknowledges these sources of variation.

In classical pharmacokinetic analysis of data from drug trials, models with relatively simple structures and few, empirical parameters are fit to data from a relatively large number of subjects, allowing for robust estimation of the distributions of those parameters. The fact that a volume of distribution, for example, would be estimated by fitting the model to the observed pharmacokinetic data was not considered a problem because the study population was assumed to be large and diverse enough to represent the ultimate target population for which predictions were desired. PBPK models have become popular in toxicology, however, because human data are rare and when available typically come from very small groups for environmental pollutants without therapeutic value. PBPK models overcome this limitation because they make use of measured values for tissue compartments and blood flows, which again are presumed to represent the larger population. Some concern arises then that updating population physiological parameters based on observations from a small sample will result in posterior distributions not truly representative of the population as a whole. Tissue volumes and partition coefficients, for example, affect model predictions and allowing them to vary can result in better model predictions than keeping them fixed, just as fitting the volume of distribution in classical pharmacokinetic models provides the flexibility to fit most data. One may question, however, whether this flexibility results in a model that is more predictive of the population as a whole and

if it masks other errors in model specification. Because of these concerns and questions, one might wish to perform analyses that treat physiological parameters as fixed while updating distributions for metabolic parameters, for which prior information is much weaker and which are known to vary considerably among individuals.

In the current study, the Monte Carlo simulation program *MCSim* (Bois and Maszle, 1998) was used to fit a PBPK model of benzene to sets of human data by performing a series of simulations along a Markov chain in the model parameter space. We hypothesized that the observed inter-individual variability resulted primarily from known or estimated variability in key metabolic parameters and that a statistical PBPK model that explicitly included variability in only those metabolic parameters (along with any known variation in body weight) would be sufficient to describe all observed variability. The result of MCMC fitting of the model to data produces samples from the Bayesian posterior distribution of the model parameters.

2. Materials and Methods

The PBPK model used in this study is based on a previously developed PBPK model for benzene metabolism in mice (Cole *et al*., 2003). The symbols and abbreviations used in the model are listed in Appendix A. The system of ordinary differential equations derived from flow-limited assumption for tissue uptake is given in Appendix B. To modify this model for risk assessment in humans, several parameters have to be adjusted. In particular, body weight (*BW*) was set at 70 kg for all individuals with the exception of the three individuals whose weights were recorded (Pekari *et al*., 1992, with personal correspondence). All fixed parameters used in the modified PBPK model can be found in Table 1 and Table 2. Total cardiac flow, Q_{Card}, and alveolar ventilation, Q_{AvV}, were assumed to be proportional to body weight and to each other. These values were defined by

$$Q_{Card} = k_{Card} \cdot BW,$$
$$Q_{AvV} = k_{QAvV} \cdot Q_{Card},$$

and the proportionality constants k_{Card} and k_{QAvV} were left for later investigation. All blood flow rates and organ volumes were changed based on reference values for a 70 kg man. Physiological values from Davies and Morris (1993) were calculated based on a reference weight of 70 kg and adjusted to satisfy the model requirement: $Q_{Card} = Q_F + Q_S + Q_R + Q_L + Q_K$. Partition constants, $P^{BZ}_{Bl:Air}$, P^{BZ}_j, and P^{BO}_j for compartments j = fat, liver, slowly perfused tissue, rapidly perfused tissue, and kidney, were also changed to adapt the model from mice to humans. The values for the concentration of microsomal protein per gram of tissue in the liver, C^{MP}, and the concentration of cytosolic protein per gram of tissue in the liver, C^{CP}, were changed from the original model and taken from Csanády *et al.* (1992). A number of metabolic rate constants were assumed to be relatively invariant between species; hence the remaining parameters in the PBPK model are unchanged from their values in Cole *et al*. (2003). In addition, the PBPK model has equations describing the cumulative amount of exhaled benzene and in order to compare the model to data of the concentration of exhaled benzene, the following expression was used to compute the model value for concentration of benzene in exhaled air

$$C^{BZ}_E = (1 - f_{alv}) \cdot C^{BZ}_I + f_{alv}[Q_{Card} \cdot (CV^{BZ} - CA^{BZ}) + Q_{AvV} \cdot C^{BZ}_I] / Q_{AvV}. \quad (3)$$

The notation from the original model is preserved in (3) with the only new value being f_{alv}, which

is the fraction of each inhaled breath that perfuses the alveolar space. This equation is essentially identical to a correction used by Jonsson and Johanson (2002) and is derived by assuming that: air leaving the alveolar region satisfies the usual venous-equilibration model; air leaving the alveolar space mixes with air that was inhaled but only entered the physiological dead space (conducting airways; DS); that the DS air does not exchange with blood at all and hence stays at the inhaled concentration; and that the measured exhaled concentration is the result of this mixture. Thus the exhaled concentration equals f_{alv} times the concentration exiting the alveolar region plus $(1 - f_{alv})$ times the DS concentration that equals the inhaled concentration. The value for f_{alv} was expected to be around 0.67 (ILSI, 1994) and was investigated as a distributional parameter with the Markov Chain Monte Carlo method. Additionally, the PBPK model has cumulative equations for urinary metabolites, and hence the model prediction of the amount of urinary metabolite was divided by a standard value of urinary excretion as converted from 20 ml/kg/day to compute the predicted concentration over time (Clark and Smith, 1983).

To illustrate the statistical considerations, suppose that a multivariate PBPK model for benzene is specified by the n-dimensional system of differential equations

$$\frac{dx}{dt} = f(t, x, q), \qquad x(t=0) = x_0. \tag{1}$$

The solution to this system of equations denoted by $g(t, q, x_0)$ is a function of parameters q (including inhalation exposure conditions), time t, and initial condition x_0. Now, consider the case of *in vivo* data collected on each of m subjects exposed to benzene. Each of these subjects are assumed to follow the basic model (1), but with potentially different parameters and initial conditions, reflecting variation in pharmacokinetic parameters across the population. Although analysis of individual subject data provides insight into underlying biology, it fails to address the broader issue of how these parameters vary across individuals. Comprehensive application of PBPK models to these data requires that both levels of inquiry, individual and population, be addressed--not only to elucidate individual-specific parameter values but also to characterize the extent and nature of their variation across population.

Formally, for individual i, with intermittent observations available at time t_{i1}, t_{i2}, … t_{in}, let $Y_{ij} = (Y_{i1j}, Y_{i2j}, \ldots, Y_{imj})^T$ be the $(m \times 1)$ vector of observations on subject i at time t_{ij}; for example, Y_{ij} may include measurements of benzene in blood and expired air. Thus, data collected on individual i are the vectors Y_{ij}, $j = 1, 2, \ldots, n_j$, ideally assumed to be observations on the system (1). However, as mentioned earlier, the measurements of f_{alv} are subject to several sources of variation. To specify this explicitly, we may specify the individual statistical model

$$Y_{ij} = g(t_{ij}, q_i, x_{0i}) + \varepsilon_{ij}, j = 1, 2, \ldots, n_i, \tag{2}$$

where ε_{ij} is a random vector representing deviations of observed data from the dynamic model due to the combined effects of these sources at time t_{ij}, and q_i and x_{01} are the parameters and initial conditions specific to individual i. Notice that the quantity of interest here is the distribution of parameters q_i.

The modified PBPK model was implemented into Frédéric Bois' and Don Maszle's Monte Carlo simulation program, *MCSim*, which uses Metropolis-Hasting sampling for its Markov Chain Monte Carlo simulations (Bois and Maszle, 1998). Markov Chain Monte Carlo simulations were run on the model in order to find distributions of specific metabolic parameters that had previously been held constant in the previous PBPK modeling studies (Cole *et al*., 2003). The model parameters investigated included V_{2E1}, the CYP2E1 specific activity as

determined by the oxidation of p-nitrophenol to p-nitrocatechol; V_{PH1} and V_{PH2}, the maximum rates of metabolism of phenol by two sulphate transferases; and V_{HQ}, the maximum rate of conjugation for hydroquinone (primarily glucuronidation). The two first order rates of metabolism of benzene oxide into phenylmercapturic acid and into muconic acid, k_3 and k_4, respectively, were also expected to be distributed and so were incorporated into the *MCSim* program. The values for k_{Card}, k_{QAvV}, and f_{alv} were likewise expected to be distributed and were analyzed using *MCSim*.

The prior distributions for V_{2E1}, V_{PH1}, V_{PH2}, and V_{HQ} were determined by analyzing previous *in vitro* data obtained from human liver samples (Seaton *et al*., 1995). The initial rates of phenol sulfation and rates of hydroquinone glucuronidation from the *in vitro* study were each multiplied by factors yielded by the mathematical model used in (Seaton *et al*., 1995). The factors were 0.18 for V_{PH1}, 2.4 for V_{PH2}, and 11.1 for V_{HQ}. The CYP2E1 activity measurements were not multiplied by a factor. These vectors of data were converted to proper units then entered in Matlab. These four vectors were tested to see if they fit a normal, uniform, gamma, or Poisson distribution. The best fitting distribution for each parameter, i.e., the hypothesized distribution that was not rejected with the highest p-value, was used as its prior. The parameters V_{2E1}, V_{PH1}, and V_{PH2} were expected to have gamma distributions based on the *in vitro* data, and the model parameter V_{HQ} was expected to be normally distributed. Since little information was available on the other investigated parameters, the remaining priors were based on previous constant values. The prior distributions for k_3 and k_4 were assumed to be normally distributed, and the means of these priors were the fixed values from the original mouse model (Cole *et al*., 2003). The priors for k_{Card}, k_{QAvV}, and f_{alv} were also assumed to be normally distributed with small standard deviations with the means of these priors originating from reference man values (ILSI, 1994). The *MCSim* program was used to find the distributional components for each of these model parameters, and the specific prior distributions used in the simulations are contained in Table 3.

Data taken from previous studies of human benzene exposure was incorporated into the *MCSim* program, and extra specifications were used in the case of multiple data sets for the same individual in order to find inter-individual variability as opposed to intra-individual variability. In one study, blood and exhaled air samples were collected from three healthy nonsmokers who were each exposed to four hour periods of both 10 cm^3/m^3 and 1.7 cm^3/m^3 benzene (Pekari *et al*., 1992). Thirty-five occupationally exposed individuals provided urine samples during their work shifts for metabolite data in a second study (Rothman *et al*., 1998 and Waidyanatha *et al*., 2004). Even though the time length of their shifts and the urine collection times varied, the exposure time for these workers was taken to be 6 hours in the model. The Markov Chain Monte Carlo simulation was run for 20,000 iterations, and the results were recorded every 10^{th} iteration. The results were analyzed from iteration 15010 through 20000 in order to ascertain the distributions of the model parameters.

Originally the *MCSim* program was run five times with five different seedings for its random number generator. The distributions resulting from these five runs were analyzed, and the output for the model parameters V_{2E1} and V_{2E1} did not have any well-fitting parametric distributions. The output for V_{2E1} varied greatly among the five runs; specifically, three of the runs yielded much higher V_{2E1} output distributions than the other two. Additionally, even when simulations were run using selective subsets of the five output distributions, the resulting model solution distributions were very narrow. In order to account for greater variability, the prior standard deviations for k_{Card} and k_{QAvV} were changed from 0.1 to 0.3 and from 0.01 to 0.05,

respectively. After this change, the *MCSim* program was run again. Only one long run was assumed to be sufficient as suggested in Geyer (1992) although Bois and Maszle believe considering several pooled runs is a better approach (Bois and Maszle, 1998).

After the posterior distributions were determined, the model was examined for sensitivity. The means of each posterior distribution were used for the investigated parameters to produce solutions from the model. In order to ascertain the model's sensitivity to each parameter, one parameter would be varied while all the other investigated parameters were kept at the mean values from their posterior distributions. For each investigated parameter, three solutions were produced holding the other eight of the parameters at their distributional means and then using a value at 95% of the confidence interval, a value at 5% of the confidence interval, and the mean of the currently analyzed parameter. Then the maximum distance from the mean solution to the solution above or below the mean curve was computed and used in the following formula

$$sensitivity = \frac{\Delta prediction \,/\, prediction}{\Delta parameter \,/\, parameter}.$$

In the above formula, *prediction* indicates the predicted solution at the mean and $\Delta prediction$ indicates the maximum difference between the predicted solution at the mean and the predicted solution using either a 95% or 5% confidence interval value. The values in the denominator of the above ratio are based on the varying investigated parameter and are defined similar to the predicted solutions. The only state variables of the model examined for sensitivity were those compared to data in this study.

3. Results

The output from *MCSim* appeared to sample adequately from the posterior distributions and was analyzed using Matlab to find distributions which best fit the output data for each parameter. These posterior distributions for the nine investigated model parameters are shown in Table 4 as well as compared graphically to their priors in Figure 1 and Figure 2. For k_{Card} the mean, μ, and standard deviation, σ, of its normal distribution are listed in the results table; and the appropriate parameters for the beta distribution are listed for f_{alv}. For the other parameters the resulting shape parameter, a, and inverse scale parameter, b, of the gamma distributions are given. The values for k_3 and k_4 are slightly below those found in the optimization with the mouse model (Cole *et al*., 2003), and the posterior distributions for the metabolic rates V_{PH1} and V_{PH2} allow for much higher values than *in vitro* data (Seaton *et al*., 1995) would suggest. The posterior distribution for V_{2E1} has moved to the left within its prior distribution, which was based on the *in vitro* data from (Seaton *et al*., 1995). Although the prior of V_{HQ} is normal and its posterior distribution appears to be a gamma distribution, the values of this parameter have changed little through the use of MCMC. The values for k_{card} and k_{QAvV} are slightly below their priors, which were based on reference mean values. The posterior distribution for f_{alv} is narrower than its prior, but the mean of the posterior is slightly higher than the mean of the prior.

One hundred solution curves were computed from the 100 samples from the *MCSim* posterior distributions and were plotted versus time along with data points from the three individuals in the (Pekari *et al*., 1992) study. These plots are contained in Figures 3-6. Here, the solution curves are plotted for each individual in the study to determine if the data points fall in the area created by the range of the solutions. The same sampling was implemented using the prior distributions to find model solutions so that the solutions based on the posterior

distributions could be compared to not only the data but also to the model prior to the use of the MCMC method. The prior distribution solutions, the posterior distribution solutions, and the data are presented on both linear and log scale to most fully represent the results. The solutions for the three individuals are plotted separately because their model solutions depended on different body weights—90 kg for subject 1, 55 kg for subject 2, and 73 kg for subject 3.

One hundred samples for each model parameter were drawn from the distributions found through *MCSim*, and 100 model solutions were computed using these parameters for different exposures using Matlab. The 100 solution values for different metabolites in the urine were plotted in Figure 7 versus the corresponding data values from the occupational exposure study (Rothman *et al*., 1998, and Waidyanatha *et al.*, 2004). Each vertical line of x's represents the 100 predicted exhaled benzene concentrations (μmoles/L) for the model for a particular inhalation concentration plotted versus an actual measurement from the study. A plot of the line $y = x$ is contained in all parts of Figure 7 for comparison. All five metabolite solutions seemed somewhat centered around the $y = x$ line except for the plot of the catechol and trihydroxy benzene concentration.

The results of the sensitivity analysis are listed in Table 5. The values in the table are based on the ratio of prediction change over parameter change as listed in the Materials and Methods section. The columns represent the parameter being changed and rows represent the different urinary metabolites and benzene blood and exhaled air concentrations evaluated for sensitivity.

4. Discussion

The model predicted metabolite data well for muconic acid, phenylmercapturic acid, and phenol and hydroquinone conjugates; but the model greatly under-predicts the concentrations of catechol and trihydroxy benzene for the workplace data (Rothman *et al*., 1998; Waidyanatha *et al.*, 2004). Since Bayesian methods depend greatly on the accuracy of prior information, small errors in the data used to estimate the priors could account for the need to alter the *MCSim* output for a better fit graphically. The model seems to predict the concentration of benzene exhaled and the concentration of benzene in blood for the (Pekari *et al.*, 1992) study well although a wider range of solution curves capturing all data points was expected. Although the computed solutions using the posterior distributions do not greatly improve accuracy over the solutions computed using the prior distributions for the this study, the range of posterior model solutions does narrow somewhat around the exposure data. Since the two studies probably varied in participant physical activity, further experiments focusing on activity levels might help the accuracy of the model.

The results for f_{alv} have a mean around 0.72, which is slightly higher than the ILSI value of 0.67 (ILSI, 1994), but the presence of benzene may have increased the subjects' ventilation rates. Measurements of the dead space lung volume of subjects exposed to butadiene suggest that it lowers the dead space to around 171.3 mL (Lin *et al*., 2001), which is around 14.6% of the total lung volume (Davies and Morris, 1993). Our results suggest that the dead space lung volume does decrease with inhaled benzene but only to about 28% of total volume. However, in the (Pekari *et al.*, 1992) study, the apparatus for sampling exhaled air may have introduced a slight source of error due to the difficulty to breathe normally. The three subjects may have unconsciously breathed with larger tidal volumes thus decreasing the relative dead space volume.

A significant portion of the data used in this investigation involved benzene concentrations in blood and exhaled air. In the PBPK model (Cole *et al*., 2003), the amount of benzene exhaled depends directly on the parameters Q_{AvV}, Q_{Card}, and $P_{Bl:Air}^{BZ}$. We have quantified Q_{AvV} and Q_{Card} as being proportional to k_{Card} and k_{QAvV}, respectively, and introduced the parameter f_{alv} for the faction of inhaled air perfusing the alveolar region. Although the equation describing AM_E^{BZ} is connected to the rest of the ordinary differential equation system, the model dynamics of the exhaled concentration of benzene are therefore most closely related to the parameters f_{alv}, k_{Card}, k_{QAvV}, and the concentration of benzene in mixed venous blood, CV^{BZ}. CV^{BZ} in turn is expected to depend strongly on the rate of clearance in the liver, which is a function blood flow to the liver (Q_L, which is proportional to k_{card}) and the rate of metabolism in the liver, particularly V_{2E1}. The results of the sensitivity analysis in Table 5 show that f_{alv}, k_{Card}, k_{QAvV}, and V_{2E1}, as well as k_3 and k_4 (rate constants for conversion of benzene oxide to phenyl-mercapturic acid and muconic acid, respectively), affect the exhaled benzene. (An increase in k_3 or k_4 reduces the amount benzene oxide which would otherwise be converted to phenol, and hence the amount of phenol competing with benzene for CYP2E1.) Not surprisingly, these same parameters, except f_{alv}, also significantly affect the predicted concentration of benzene in blood.

Since the predictions of benzene concentrations in exhaled air and in blood depend most strongly on k_3, k_4, V_{2E1}, f_{alv}, k_{card}, and k_{QAvV}, *MCSim* would primarily be able affect the fit of the model to the exhalation and blood data by updating the distributions of these particular six parameters. Hence, the posterior distributions found by *MCSim* for k_3, k_4, V_{2E1}, f_{alv}, k_{Card}, and k_{QAvV} were largely influenced by the process of fitting solution curves to the exhalation and blood data. Likewise, since changes in f_{alv} only alter the exhaled benzene predictions, only the exhalation data affects the f_{alv} posterior distribution in the MCMC simulations. The model does not seem to be very sensitive to changes in V_{PH1}, V_{PH2}, and V_{HQ}, although the effect is slightly higher when dealing with the metabolites. The sensitivity of model predictions for urinary metabolite concentrations to changes in k_3, k_4, V_{2E1}, V_{PH1}, V_{PH2}, V_{HQ}, k_{Card}, and k_{QAvV} suggests that the data from the occupational study influences the distributions estimated for all investigated parameters except for f_{alv}.

When the model prediction of one output variable is less sensitive to the change in a particular parameter, the *MCSim* program will be more influenced by the data of other output variables (that do show greater sensitivity) when finding the posterior distribution of that particular parameter. Hence, the effect the urinary metabolite data has on the distributions of V_{PH1}, V_{PH2}, and V_{HQ} is less than the occupational study data has on other parameters and is less significant than the effect the blood and exhalation data have on most other parameters. Further, any miscalculation due to the apparatus used by Pekari *et al.* (1992) will significantly affect our results for f_{alv}, and use of the data from Rothman *et al*. (1998) and (Waidyanatha *et al.* (2004) cannot significantly compensate for such an error. Similarly, the results for V_{PH1} and V_{PH2} are primarily determined by metabolite data, but errors here seem not to greatly affect the model overall. Using a variety of types of data should result in better estimates of parameter distributions, but understanding that certain data sets are more critical than others in the determination of each model parameter is also critical in developing the most accurate model.

From a statistical viewpoint, when both the mathematical model and the statistical model are fully specified at all levels, the approach is referred to as a parametric approach. Specifically in the PBPK model, the approach is considered parametric when assuming a distribution form (e.g., log-normal) for how well we know each individual's parameters and a distribution form for the set of parameters from all the individuals. A parametric approach is often used when the

general form of the distribution in the problem is known. Second, a statistical model is considered to be structured when the variables (distributions) are associated with specific underlying quantities, which occurs naturally with PBPK model. A parametric approach provides an efficient way for estimating the parameters of a PBPK model since it takes full advantage of the distribution structure.

A variety of statistical tools are available for fitting the mathematical models to data with structured variability. Model-fitting tools that do not incorporate prior information on parameter distributions, referred to as "frequentist," include maximum likelihood methods such as non-linear least squares (Bates and Watts, 1988). The error structure in the data (how sources of error or variability are assigned) can be accommodated by modifying standard techniques to incorporate ideas from repeated measures and cross-over experimental designs. Alternatively, Bayesian statistical models provide a natural framework for analyzing models with hierarchical error structures (Gelman *et al*. 1996). One Bayesian technique that has been embraced by a great many applied statisticians in all fields of research is the Markov chain Monte Carlo (MCMC) method (Gilks *et al*., 1996). MCMC methods explore the joint posterior distribution of interest (i.e., the distribution of all parameters, given that the distributions may not be independent) by providing a mechanism whereby a set of realizations or samples from that distribution can be generated. This set is obtained by carrying out Monte Carlo simulations from a Markov chain that is constructed so that its stationary distribution is the relevant posterior. Various methodologies exist to carry out the required simulations including the Gibbs sampling algorithm and the Metropolis-Hastings algorithm. Because of the increasing complexities of statistical models encountered in practice, MCMC provides a much-needed unifying framework within which many complex problems can be analyzed.

Both Bayesians and frequentists need to integrate over possibly high-dimensional probability distributions, such as unknown parameters, to make inferences for the parameter of interest or to make predictions. This basic need underlies the potential role of MCMC methodology in statistical modeling and inference. The past several years have witnessed an explosive growth of interest in MCMC methodology from researchers in almost all areas of statistics and biology. In particular, Bayesian population methods have made some significant contributions in the field of PBPK modeling. In (Bois *et al.*, 1996) Bayesian statistical inference and physiological modeling were brought together to model the distribution and metabolism of benzene in humans. This approach of combining PBPK models and MCMC methodology for Bayesian inference has been extended to other chemicals such as toluene and styrene (Jonsson and Johanson, 2001; 2002). The inclusion of the variability predicted by these approaches into risk assessments is expected to be an improvement over previous use of empirical uncertainty factors (e.g., Lipscomb *et al.*, 2003).

Bois *et al.* (1996) also applied Bayesian analysis to a PBPK model of benzene in humans, using the data of Pekari *et al.* (1992), which is also included in our analysis. The paper of Bois and co-workers was one of the first to demonstrate the application of Bayesian analysis to PBPK modeling and its use in predicting population variability, a significant advancement in the potential for mechanistic dosimetry modeling in risk analysis. The model used by Bois *et al*., however, only included a very simple description of benzene metabolites, with the assumption that phenolic metabolites are a fixed fraction of those metabolites. That model does not allow for prediction of target tissue concentrations of phenol itself (as opposed to phenol conjugates), nor of hydroquinone or benzene oxide, both of which are included in our model. Hydroquinone and phenol have been shown to strongly synergize in the induction of genotoxicity *in vivo*

(Barale *et al.*, 1990) and hydroquinone was shown to strongly enhance colony formation of murine bone marrow cells *in vitro* (Irons *et al.*, 1992). Benzene oxide has been shown to be tumorigenic in mice (Busby *et al.*, 1990) and benzene exposure-related increases in benzene oxide-albumin adducts have been demonstrated in humans (Rappaport *et al.*, 2002). Thus we believe the current model builds upon and is a considerable advancement of the innovative work by Bois and colleagues in that it predicts tissue levels of phenol, hydroquinone, and benzene oxide, all of which are likely contributors to benzene's leukemogenic effects. Further, the current results are based on a much larger data set than that used in the previous analysis, providing for more robust and representative posterior distributions.

A known source of variability that has been better accounted for elsewhere is the effect of activity level on circulation and respiration (Jonsson and Johanson, 2001, 2002). Not only were we faced with a lack of data on activity level among the subjects in the studies included here, but also we were working with many more metabolic parameters for which the priors are not strongly informative. However, the idea that the effect of known or estimated variability in these metabolic parameters could more than cover the observed variability in the data available seemed possible. Therefore we decided to test the hypothesis that the observed variability in the data would be accounted for by incorporating distributions for *only* the metabolic parameters by fixing the values of most physiological variables to standard values used in PBPK modeling (e.g., ILSI, 1994) and the measured partition coefficients to those measured or estimated elsewhere.

Incorporation of dependence on activity level and variability and uncertainty in physiological parameters and partition coefficients would almost certainly have resulted in much closer correlations between predictions and the data and should probably be added before the benzene PBPK model is used in a human risk assessment, but we believe there is scientific value in first testing this more stringent assumption presented here. While we do not know precise activity levels for the individuals whose data are being simulated here, Jonsson and Johanson (2001) found the best description of their data for toluene when "the increased perfusion of peri-renal fat was set to a constant level during all exercise levels," which indicates that categorical assignment of values based on general activity patterns (e.g., resting, some movement, light work, etc.) would be sufficient.

At the beginning of this study, additional data was considered from Berlin *et al*. (1980), who measured benzene concentrations in exhaled breath after inhalation exposures. Results reported by Berlin *et al*. were different enough from those of Pekari *et al.* (1992) that including both sets of data in our analysis became problematic. Since the Pekari *et al*. data seemed more informative, our attempt to include the Berlin data was abandoned. In early studies incorporating the Berlin data, the model fits of benzene concentration in blood and exhaled air to the data of Pekari data were fairly good and appeared to be without significant bias. At least in those cases the model predicted the relationship between blood concentrations and exhaled air concentrations. Recognizing that the Berlin *et al.* (1980) and Waidyanatha *et al.* (2004) data sets are from distinct populations, we note that there is a tendency for the model to over-predict both of those; i.e., it seems to over-predict *both* the amount in exhaled breath and urinary excretion when the Berlin data is incorporated into the study. Since any benzene inhaled must either be excreted by one of these routes or bind to tissue macromolecules (first order rates from phenol and hydroquinone), the only way to decrease both exhaled breath predictions *and* urinary excretion predictions would be to either increase the amount predicted as binding to macromolecules or decrease the predicted inhalation rate. Since we believe the rate constants for

binding to macromolecules to represent non-enzymatic reactions that should be independent of species, their values were not updated from those estimated by Cole *et al.* (2003) using mouse *in vivo* data.

Thus we could potentially "correct" the over-prediction of the Berlin *et al.* (1980) data and Waidyanatha *et al.* (2004) data by updating k_9 and k_{10}. If we had allowed those parameters to be updated better fits probably could have been obtained without further insight. Instead the failure of the model to fit the data given the constraints of holding those parameters constants led us to the possibility that we had over-predicted the rate of uptake by inhalation. Benzene has a blood:air partition coefficient of 7.8 (Brown *et al.*, 1998). While this is relatively low and benzene has low aqueous solubility, one might expect a limited wash-in/wash-out effect that would lower absorption from the amount predicted by the classic venous ventilation model used here (see Gerde and Dahl, 1991). After this analysis, we decided not to use the data from Berlin *et al*. (1980) in our study rather than alter k_9 or k_{10} or the blood:air partition coefficient. In addition to not utilizing the Berlin data, we did update the cardiac flow and alveolar ventilation rate through the parameters k_{Card} and k_{QAvV}. After the decision to neglect the data from Berlin *et al*. (1980) in our analysis, k_9 and k_{10} were investigated briefly through the MCMC method using priors based on values from Cole *et al.* (2003). The intention was to improve the results with catechol and tryhydroxy benzene, but no significant improvement resulted in the fit to data from either the Pekari *et al.* (1992) study or the occupational data from Rothman *et al*. (1998) and Waidyanatha *et al.* (2004).

The difference between a model adjustment by increasing the macromolecule binding constants and decreasing predicted inhalation rates is potentially significant because the later would result in a reduction in the prediction of total phenol and hydroquinone production and hence in potential target tissue dosimetry of active metabolites. Future work on benzene PBPK modeling for humans should probably first seek to implement a more anatomically accurate inhalation model, such as those described by Sarangapani *et al.* (2002) or Csanady *et al.* (2003) before updating the macromolecule binding constants. If after making such structural changes to eliminate model bias the predicted pharmacokinetic distributions still do not cover the data, we would then have greater support for including other sources of variability and possibly updating their distributions as well.

Given the constraints of fixing many model parameters to standard or previously reported values, the fact that it does a fairly good job of describing the data from two different studies suggests that the model structure is essentially sound and affirms the use of key parameters from *in vitro* or laboratory animal studies. It is fortunate that we have a number of human data for benzene to use in parameter estimation and demonstrate model quality. However these results should also support similar *in vitro* to *in vivo* and animal to human extrapolations through PBPK modeling for other compounds with fewer or no human data available.

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Table 1: Fixed parameters used in the PBPK model (Cole, *et al.*, 2003)

Parameter	Value	Unit	Source
Q_L	$0.2370Q_{Card}$	L/hr	(Davies and Morris, 1993)
Q_F	$0.0425Q_{Card}$	L/hr	(Davies and Morris, 1993)
Q_K	$0.2027Q_{Card}$	L/hr	(Davies and Morris, 1993)
Q_S	$0.1717Q_{Card}$	L/hr	(Davies and Morris, 1993)
Q_R	$0.3461Q_{Card}$	L/hr	(Davies and Morris, 1993)
V_L	$0.025BW$	L	(ILSI, 1994)
V_F	$0.1429BW$	L	(Davies and Morris, 1993)
V_K	$0.004BW$	L	(Davies and Morris, 1993)
V_S	$0.734BW$	L	(ILSI, 1994)
V_R	$0.040BW$	L	(ILSI, 1994)
V_{Bl}	$0.07429BW$	L	(Davies and Morris, 1993)
C^{CP}	14.5	mg/g	(Csanady *et al.*, 1992)
C^{MP}	58	mg/g	(Csanady *et al.*, 1992)
$K_{m,1}^{PH}$	1.4	μM	(Seaton *et al.*, 1995)
$K_{m,2}^{PH}$	220	μM	(Seaton *et al.*, 1995)
K_m^{HQ}	746	μM	(Seaton *et al.*, 1995)
A^{BZ}	0.0397	1/μM	(Lovern *et al.*, 1999)
A^{PH}	$1.30 \cdot 10^{-2}$	1/μM	(Lovern *et al.*, 1999)
A^{HQ}	10^{-7}	1/μM	(Lovern *et al.*, 1999)
k_1	$4.20 \cdot 10^{-2}$	L/μmol	(Lovern *et al.*, 1999)
k_2	32.16	1/hr	(Lovern *et al.*, 1999)
k_5	$4.00 \cdot 10^{-2}$	L/μmol	(Lovern *et al.*, 1999)
k_6	$2.13 \cdot 10^{-3}$	L/μmol	(Lovern *et al.*, 1999)
k_7	$2.03 \cdot 10^{-4}$	L/μmol	(Lovern *et al.*, 1999)
k_8	374.9598	1/hr	(Cole *et al.*, 2003)
k_9	0.1163	1/hr	(Cole *et al.*, 2003)
k_{10}	0.1443	1/hr	(Cole *et al.*, 2003)

Table 2: Partition coefficients used in the PBPK model (Cole, *et al.*, 2003)

Parameter	Value	Source
$P_{Bl:Air}^{BZ}$	7.80	(Brown *et al.*, 1998)
P_F^{BZ}, P_F^{BO}	54.50	(Brown *et al.*, 1998)
P_L^{BZ}, P_L^{BO}	2.95	(Brown *et al.*, 1998)
P_S^{BZ}, P_S^{BO}	2.05	(Brown *et al.*, 1998)
$P_R^{BZ}, P_R^{BO}, P_K^{BZ}, P_K^{BO}$	1.92	(Brown *et al.*, 1998)
P_F^{PH}	27.63	(Leung *et al.*, 1990)
P_L^{PH}	2.17	(Leung *et al.*, 1990)
P_S^{PH}	1.22	(Leung *et al.*, 1990)
P_R^{PH}, P_K^{PH}	2.17	(Leung *et al.*, 1990)
P_F^{HQ}	4.06	(Leung *et al.*, 1990)
P_L^{HQ}	1.04	(Leung *et al.*, 1990)
P_S^{HQ}	0.94	(Leung *et al.*, 1990)
P_R^{HQ}, P_K^{HQ}	1.04	(Leung *et al.*, 1990)

Table 3: Prior distributions for the PBPK model parameters analyzed using the Markov Chain Monte Carlo Method.

Parameter	Prior Distribution
k_3	Normal, μ=0.7032, σ=0.1
k_4	Normal, μ=15.1001, σ=1
V_{2E1}	Gamma, a=2.7506, b=0.0284
V_{PH1}	Gamma, a=6.8926, b=0.0044
V_{PH2}	Gamma, a=6.8926, b=0.0585
V_{HQ}	Normal, μ=0.7484, σ=0.3207
f_{alv}	Normal, μ=0.67, σ=0.2
k_{Card}	Normal, μ=4.4571, σ=0.3
$k_{Q_{AvV}}$	Normal, μ=0.965, σ=0.05

Table 4: The resulting distributions for the PBPK model parameters from the Markov Chain Monte Carlo Method.

Parameter	Posterior Distribution
k_3	Gamma, a=63.8027, b=$8.060 \cdot 10^{-3}$
k_4	Gamma, a=170.74, b=0.07282
V_{2E1}	Gamma, a=140.58, b=$1.286 \cdot 10^{-4}$
V_{PH1}	Gamma, a=7.310, b=225.53
V_{PH2}	Gamma, a=7.8377, b=15.5516
V_{HQ}	Gamma, a=20.2275, b=0.05037
f_{alv}	Beta, a=169.29, b=65.7663
k_{Card}	Normal, μ=3.2635, σ=0.2634
$k_{Q_{AvV}}$	Gamma, a=276.63, b=$2.557 \cdot 10^{-3}$

Table 5: The sensitivity analysis results for each investigated parameter.

	k_3	k_4	V_{2E1}	V_{PH1}	V_{PH2}	V_{HQ}	f_{alv}	k_{Card}	$k_{Q_{AvV}}$
MA	2.07	1.65	2.20	$6.9\cdot10^{-4}$	0.058	0.21	0	2.30	4.40
Cat-THB	1.66	2.00	2.18	$5.6\cdot10^{-4}$	0.014	0.13	0	1.51	3.89
PMA	0.99	2.04	1.98	$5.9\cdot10^{-4}$	0.015	0.17	0	2.33	4.23
PH	2.40	0.86	2.58	$7.8\cdot10^{-4}$	0.020	0.23	0	6.33	3.91
HQ	0.59	0.91	1.47	$2.1\cdot10^{-4}$	0.0053	0.67	0	0.69	1.51
C_E^{BZ} (high)	2.03	3.90	2.24	$2.3\cdot10^{-6}$	$3.2\cdot10^{-6}$	$4.2\cdot10^{-5}$	1.94	3.21	3.09
C_E^{BZ} (low)	0.089	1.56	2.01	$5.0\cdot10^{-6}$	$2.4\cdot10^{-5}$	$1.7\cdot10^{-7}$	1.94	4.55	3.63
$CA^{BZ}+CV^{BZ}$ (high)	2.03	3.90	2.24	$2.3\cdot10^{-6}$	$3.2\cdot10^{-6}$	$4.2\cdot10^{-5}$	0	3.21	3.15
$CA^{BZ}+CV^{BZ}$ (low)	0.089	1.56	2.01	$5.0\cdot10^{-6}$	$2.4\cdot10^{-5}$	$1.7\cdot10^{-7}$	0	4.55	3.60

Appendix A: Model symbols

The following symbols and abbreviations are used in the PBPK model given in appendix B. Units of the symbols are given in parentheses.

Chemical Abbreviations:

BZ	Benzene
BO	Benzene oxide
PH	Phenol
HQ	Hydroquinone
MA	Muconic acid
PMA	Phenylmercapturic acid
PH-Conj	Phenol conjugates
HQ-Conj	Hydroquinone conjugates
Cat	Catechol
THB	Trihydroxy benzene

Compartment Abbreviations:

F	Fat
S	Slowly or Poorly Perfused Tissue
R	Rapidly or Richly Perfused Tissue
K	Kidney
L1	Zone 1 of the Liver
L2	Zone 2 of the Liver
L3	Zone 3 of the Liver
Bl	Blood
Stom	Stomach
I	Inhaled air
E	Exhaled air

Primary Symbols:

C_j^i	Concentration of chemical i in tissue j (μmol/L)
CA^{BZ}	Concentration of *BZ* in the arterial blood (μmol/L)
CV^{BZ}	Concentration of *BZ* in the venous blood (μmol/L)
CV_j^i	Concentration of chemical i in venous blood from tissue j (μmol/L)
C_I^{BZ}	Concentration of *BZ* in inhaled air (μmol/L)
C_E^{BZ}	Concentration of *BZ* in exhaled air (μmol/L)
AM^i	Amount of chemical i in urine (μmol)
AM^{Stom}	Amount of *BZ* in the stomach (μmol)
RM_j^i	Rate of metabolism of chemical i to chemical j (μmol/hr)
Q_j	Flow in tissue j (L/hr)

Q_{AvV}	Alveolar ventilation (L/hr)
Q_{Card}	Cardiac blood output (L/hr)
P_j^i	Tissue *j*/blood partition coefficient for chemical i
$P_{Bl:Air}^{BZ}$	Blood/air partition coefficient for *BZ*
BW	Body weight (kg)
V_j	Volume of tissue *j* (L)
T_L	Total mass of the liver (g)
C^{MP}	Concentration of microsomal protein per gram of tissue in the liver (mg/g)
C^{CP}	Concentration of cytosolic protein per gram of tissue in the liver (mg/g)
V_{2E1}	CYP2E1 specific activity as determined by the oxidation of p-nitrophenol to p-nitrocatechol (μmol/mg/hr)
A^i	Affinity parameter for CYP2E1 for substrate i (L/μmol)
$k_1, k_5 - k_7$	Efficiencies of CYP2E1 for specific oxidation relative to V_{2E1} (L/μmol)
$k_2 - k_4$	First-order rates of metabolism (1/hr)
k_8	Rate of uptake from the stomach to the liver (1/hr)
k_9, k_{10}	Binding coefficients (1/hr)
V_{PH1}, V_{PH2}	Maximum rates of metabolism of *PH* by two sulfate transferases (μmol/mg/hr)
$K_{m,1}^{PH}, K_{m,2}^{PH}$	Concentrations at half-saturation of *PH* by two sulfate transferases (μmol/L)
V_{HQ}	Maximum rate of metabolism for *HQ* (μmol/mg/hr)
K_m^{HQ}	Concentration at half-saturation for *HQ* (μmol/L)
k_{Card}	Proportionality constant between *BW* and Q_{Card} (L/(h*kg))
k_{QAvV}	Proportionality constant between Q_{Card} and Q_{AvV} (unit-less)
f_{alv}	Fraction of each inhaled breath that perfuses the alveolar space (unit-less)

Appendix B: Mathematical Model

The following system of ordinary differential equations that was derived in (Cole, et al., 2003) was based on a perfusion-limited model, or equivalently, a flow-limited model of disposition. More specifically, it was assumed that the rate of uptake of benzene into a tissue compartment is limited by the blood flow rate to the tissue rather than the rate of diffusion across the cell membrane. For the sake of completeness the model equations and the differential equations (grouped by chemical) are given below.

Explicit Equations

Concentration of chemical i in venous blood leaving compartment j: $CV_j^i = \dfrac{C_j^i}{P_j^i}$

Cardiac flow: $Q_{Card} = Q_F + Q_S + Q_R + Q_L + Q_K$

Concentration of BZ in venous blood:

$$CV^{BZ} = \frac{CV_F^{BZ} Q_F + CV_S^{BZ} Q_S + CV_R^{BZ} Q_R + CV_L^{BZ} Q_L + CV_K^{BZ} Q_K}{Q_{Card}}$$

Concentration of BZ in arterial blood: $CA^{BZ} = \frac{Q_{AvV} C_I^{BZ} + Q_{Card} CV^{BZ}}{\frac{Q_{AvV}}{P_{Bl:Air}} + Q_{Card}}$

CYP2E1 activity in the liver:

$$RM_{BO,L3}^{BZ} = k_1 \frac{V_{2E1} C_{L3}^{BZ}}{D_L} C^{MP} \frac{T_L}{3}$$

$$RM_{HQ,L3}^{PH} = k_5 \frac{V_{2E1} C_{L3}^{PH}}{D_L} C^{MP} \frac{T_L}{3}$$

$$RM_{Cat,L3}^{PH} = k_6 \frac{V_{2E1} C_{L3}^{PH}}{D_L} C^{MP} \frac{T_L}{3}$$

$$RM_{THB,L3}^{HQ} = k_7 \frac{V_{2E1} C_{L3}^{HQ}}{D_L} C^{MP} \frac{T_L}{3}$$

$$D_L = 1 + A^{BZ} C_{L3}^{BZ} + A^{PH} C_{L3}^{PH} + A^{HQ} C_{L3}^{HQ}$$

CYP2E1 activity in the kidney:

$$RM_{BO,K}^{BZ} = k_1 \frac{V_{2E1}}{10} \frac{C_K^{BZ}}{D_K} C^{MP} T_K$$

$$RM_{HQ,K}^{PH} = k_5 \frac{V_{2E1}}{10} \frac{C_K^{PH}}{D_K} C^{MP} T_K$$

$$RM_{Cat,K}^{PH} = k_6 \frac{V_{2E1}}{10} \frac{C_K^{PH}}{D_K} C^{MP} T_K$$

$$RM_{THB,K}^{HQ} = k_7 \frac{V_{2E1}}{10} \frac{C_K^{HQ}}{D_K} C^{MP} T_K$$

$$D_K = 1 + A^{BZ} C_K^{BZ} + A^{PH} C_K^{PH} + A^{HQ} C_K^{HQ}$$

Total mass of j=liver or kidney: $T_j = V_j * \frac{10^3 g}{1L}$

Metabolism of BO to PH in compartment j: $RM_{PH,j}^{BO} = k_2 C_j^{BO} V_j$

Metabolism of BO to PMA in compartment j: $RM_{PMA,j}^{BO} = k_3 C_j^{BO} V_j$

Metabolism of BO to MA in compartment j: $RM_{MA,L3}^{BO} = k_4 C_{L3}^{BO} \frac{V_L}{3}$

Conjugation of PH: $RM_{Conj,L1}^{PH} = \left(\frac{V_{PH1} C_{L1}^{PH}}{K_{m,1}^{PH} + C_{L1}^{PH}} + \frac{V_{PH2} C_{L1}^{PH}}{K_{m,2}^{PH} + C_{L1}^{PH}} \right) C^{CP} \frac{T_L}{3}$

Conjugation of HQ: $RM_{Conj,L3}^{HQ} = \frac{V_{HQ} C_{L3}^{HQ}}{K_m^{HQ} + C_{L3}^{HQ}} C^{MP} \frac{T_L}{3}$

Concentration of exhaled benzene:

$$C_E^{BZ} = (1 - f_{alv}) \cdot C_I^{BZ} + f_{alv} [Q_{Card} \cdot (CV^{BZ} - CA^{BZ}) + Q_{AvV} \cdot C_I^{BZ}] / Q_{AvV}$$

Benzene

Fat: $V_F \frac{dC_F^{BZ}}{dt} = Q_F (CA^{BZ} - CV_F^{BZ})$

Slowly: $V_S \frac{dC_S^{BZ}}{dt} = Q_S (CA^{BZ} - CV_S^{BZ})$

Rapidly: $V_R \frac{dC_R^{BZ}}{dt} = Q_R (CA^{BZ} - CV_R^{BZ})$

Kidney: $V_K \frac{dC_K^{BZ}}{dt} = Q_K (CA^{BZ} - CV_K^{BZ}) - RM_{BO,K}^{BZ}$

Liver (Zone 1): $\frac{V_L}{3} \frac{dC_{L1}^{BZ}}{dt} = Q_L (CA^{BZ} - C_{L1}^{BZ}) + k_8 AM^{Stom}$

Liver (Zone 2): $\frac{V_L}{3} \frac{dC_{L2}^{BZ}}{dt} = Q_L (C_{L1}^{BZ} - C_{L2}^{BZ})$

Liver (Zone 3): $\frac{V_L}{3} \frac{dC_{L3}^{BZ}}{dt} = Q_L (C_{L2}^{BZ} - CV_{L3}^{BZ}) - RM_{BO,L3}^{BZ}$

Stomach: $\frac{dAM^{Stom}}{dt} = -k_8 AM^{Stom}$

Amount Exhaled: $\frac{dAM_E^{BZ}}{dt} = Q_{Card} (CV^{BZ} - CA^{BZ}) + Q_{AvV} \cdot C_I^{BZ}$

Benzene Oxide

Blood: $V_{Bl} \frac{dC_{Bl}^{BO}}{dt} = Q_F CV_F^{BO} + Q_S CV_S^{BO} + Q_R CV_R^{BO} + Q_K CV_K^{BO}$
$+ Q_L CV_{L3}^{BO} - Q_{Card} C_{Bl}^{BO} - RM_{PMA,Bl}^{BO} - RM_{PH,Bl}^{BO}$

Fat: $V_F \frac{dC_F^{BO}}{dt} = Q_F (C_{Bl}^{BO} - CV_F^{BO}) - RM_{PMA,F}^{BO} - RM_{PH,F}^{BO}$

Slowly: $V_S \frac{dC_S^{BO}}{dt} = Q_S (C_{Bl}^{BO} - CV_S^{BO}) - RM_{PMA,S}^{BO} - RM_{PH,S}^{BO}$

Rapidly: $V_R \frac{dC_R^{BO}}{dt} = Q_R (C_{Bl}^{BO} - CV_R^{BO}) - RM_{PMA,R}^{BO} - RM_{PH,R}^{BO}$

Kidney: $V_K \frac{dC_K^{BO}}{dt} = Q_K(C_{Bl}^{BO} - CV_K^{BO}) + RM_{BO,K}^{BZ} - RM_{PH,K}^{BO} - RM_{PMA,K}^{BO}$

Liver (Zone1): $\frac{V_L}{3}\frac{dC_{L1}^{BO}}{dt} = Q_L(C_{Bl}^{BO} - C_{L1}^{BO}) - RM_{PH,L1}^{BO}$

Liver (Zone 2): $\frac{V_L}{3}\frac{dC_{L2}^{BO}}{dt} = Q_L(C_{L1}^{BO} - C_{L2}^{BO}) - RM_{PH,L2}^{BO}$

Liver (Zone 3): $\frac{V_L}{3}\frac{dC_{L3}^{BO}}{dt} = Q_L(C_{L2}^{BO} - CV_{L3}^{BO}) + RM_{BO,L3}^{BZ} - RM_{PH,L3}^{BO} - RM_{MA,L3}^{BO} - RM_{PMA,L3}^{BO}$

Muconic Acid

$$\frac{dAM^{MA}}{dt} = RM_{MA,L3}^{BO}$$

Phenylmercapturic Acid

$$\frac{dAM^{PMA}}{dt} = RM_{PMA,Bl}^{BO} + RM_{PMA,F}^{BO} + RM_{PMA,S}^{BO} + RM_{PMA,R}^{BO} + RM_{PMA,K}^{BO} + RM_{PMA,L3}^{BO}$$

Phenol

Blood: $V_{Bl}\frac{dC_{Bl}^{PH}}{dt} = Q_F CV_F^{PH} + Q_S CV_S^{PH} + Q_R CV_R^{PH} + Q_K CV_K^{PH} + Q_L CV_{L3}^{PH} - Q_{Card} C_{Bl}^{PH} + RM_{PH,Bl}^{BO} - k_9 C_{Bl}^{PH} V_{Bl}$

Fat: $V_F \frac{dC_F^{PH}}{dt} = Q_F(C_{Bl}^{PH} - CV_F^{PH}) + RM_{PH,F}^{BO} - k_9 C_F^{PH} V_F$

Slowly: $V_S \frac{dC_S^{PH}}{dt} = Q_S(C_{Bl}^{PH} - CV_S^{PH}) + RM_{PH,S}^{BO} - k_9 C_S^{PH} V_S$

Rapidly: $V_R \frac{dC_R^{PH}}{dt} = Q_R(C_{Bl}^{PH} - CV_R^{PH}) + RM_{PH,R}^{BO} - k_9 C_R^{PH} V_R$

Kidney: $V_K \frac{dC_K^{PH}}{dt} = Q_K(C_{Bl}^{PH} - CV_K^{PH}) + RM_{PH,K}^{BO} - RM_{HQ,K}^{PH} - RM_{Cat,K}^{PH} - k_9 C_K^{PH} V_K$

Liver (Zone 1): $\frac{V_L}{3}\frac{dC_{L1}^{PH}}{dt} = Q_L(C_{Bl}^{PH} - C_{L1}^{PH}) + RM_{PH,L1}^{BO} - RM_{Conj,L1}^{PH} - k_9 C_{L1}^{PH}\frac{V_L}{3}$

Liver (Zone 2): $\frac{V_L}{3}\frac{dC_{L2}^{PH}}{dt} = Q_L(C_{L1}^{PH} - C_{L2}^{PH}) + RM_{PH,L2}^{BO} - k_9 C_{L2}^{PH}\frac{V_L}{3}$

Liver (Zone 3): $$\frac{V_L}{3}\frac{dC_{L3}^{PH}}{dt} = Q_L(C_{L2}^{PH} - CV_{L3}^{PH}) + RM_{PH,L3}^{BO} - RM_{HQ,L3}^{PH} - RM_{Cat,L3}^{PH} - k_9 C_{L3}^{PH}\frac{V_L}{3}$$

Phenol Conjugates

$$\frac{dAM^{PH-Conj}}{dt} = RM_{Conj,L1}^{PH}$$

Hydroquinone

Blood: $$V_{Bl}\frac{dC_{Bl}^{HQ}}{dt} = Q_F CV_F^{HQ} + Q_S CV_S^{HQ} + Q_R CV_R^{HQ} + Q_K CV_K^{HQ} + Q_L CV_{L3}^{HQ} - Q_{Card} C_{Bl}^{HQ} - k_{10} C_{Bl}^{HQ} V_{Bl}$$

Fat: $$V_F\frac{dC_F^{HQ}}{dt} = Q_F(C_{Bl}^{HQ} - CV_F^{HQ}) - k_{10} C_F^{HQ} V_F$$

Slowly: $$V_S\frac{dC_S^{HQ}}{dt} = Q_S(C_{Bl}^{HQ} - CV_S^{HQ}) - k_{10} C_S^{HQ} V_S$$

Rapidly: $$V_R\frac{dC_R^{HQ}}{dt} = Q_R(C_{Bl}^{HQ} - CV_R^{HQ}) - k_{10} C_R^{HQ} V_R$$

Kidney: $$V_K\frac{dC_K^{HQ}}{dt} = Q_K(C_{Bl}^{HQ} - CV_K^{HQ}) + RM_{HQ,K}^{PH} - RM_{THB,K}^{HQ} - k_{10} C_K^{HQ} V_K$$

Liver (Zone 1): $$\frac{V_L}{3}\frac{dC_{L1}^{HQ}}{dt} = Q_L(C_{Bl}^{HQ} - C_{L1}^{HQ}) - k_{10} C_{L1}^{HQ}\frac{V_L}{3}$$

Liver (Zone 2): $$\frac{V_L}{3}\frac{dC_{L2}^{HQ}}{dt} = Q_L(C_{L1}^{HQ} - C_{L2}^{HQ}) - k_{10} C_{L2}^{HQ}\frac{V_L}{3}$$

Liver (Zone 3): $$\frac{V_L}{3}\frac{dC_{L3}^{HQ}}{dt} = Q_L(C_{L2}^{HQ} - CV_{L3}^{HQ}) + RM_{HQ,L3}^{PH} - RM_{THB,L3}^{HQ} - RM_{Conj,L3}^{HQ} - k_{10} C_{L3}^{HQ}\frac{V_L}{3}$$

Hydroquinone Conjugates

$$\frac{dAM^{HQ-Conj}}{dt} = RM_{Conj,L3}^{HQ}$$

Catechol and Trihydroxy benzene

$$\frac{dAM^{Cat/THB}}{dt} = RM_{Cat,L3}^{PH} + RM_{Cat,K}^{PH} + RM_{THB,L3}^{HQ} + RM_{THB,K}^{HQ}$$

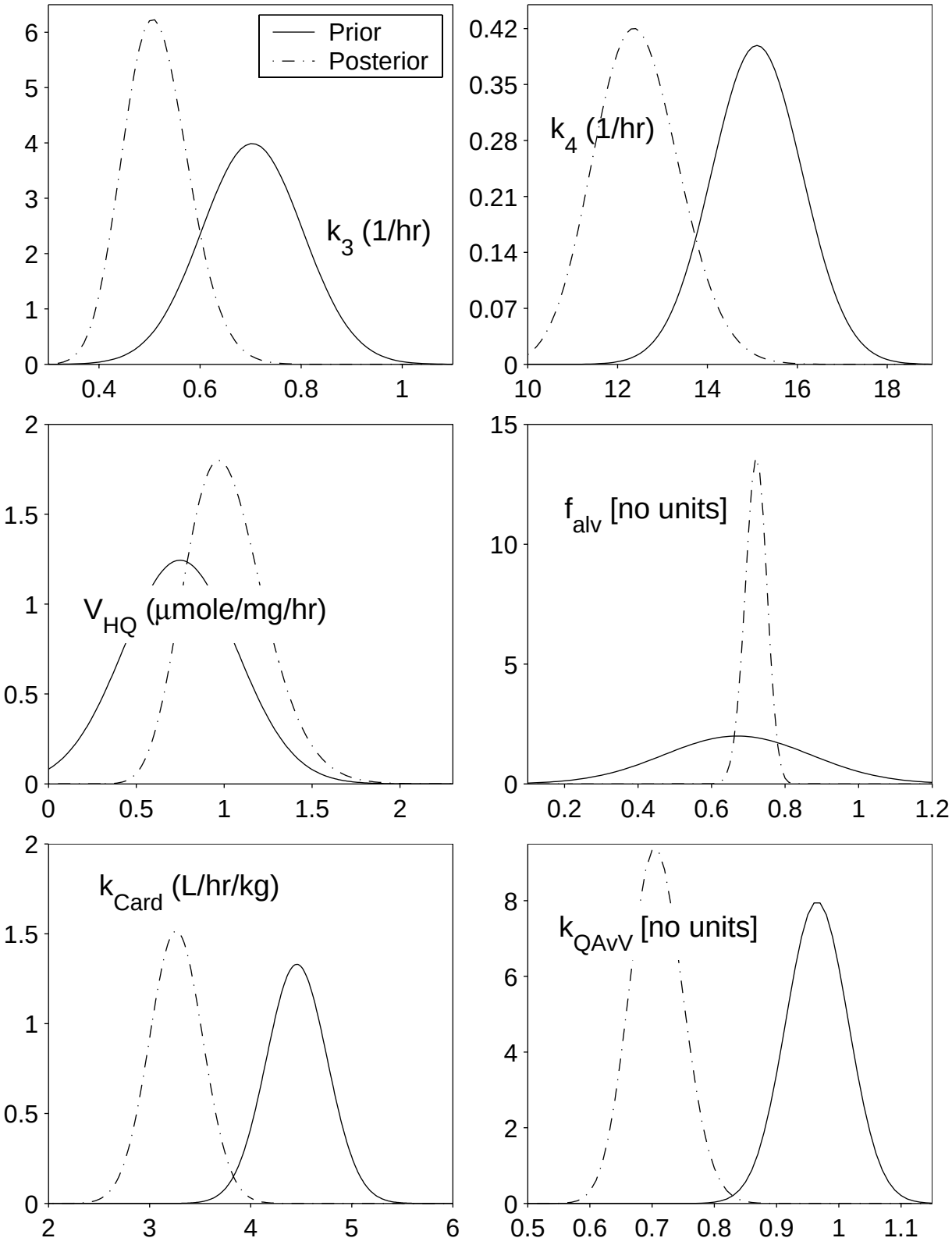

Figure 1: Prior distributions plotted alongside posterior distributions for six of the investigated parameters.

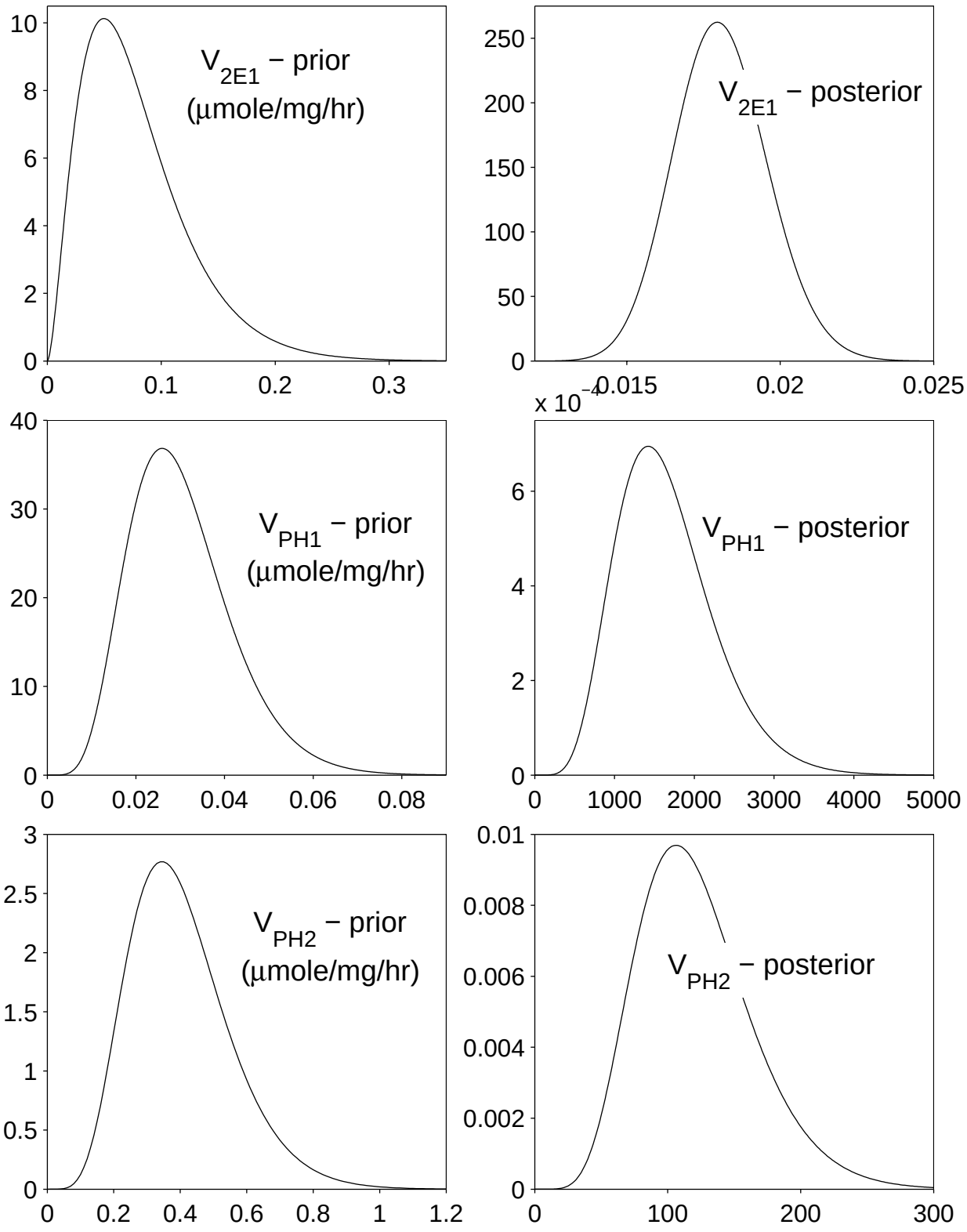

Figure 2: Prior distributions and posterior distributions for three of the investigated parameters. Note that the different distributions prevented plotting the prior and posterior of each of these parameters on the same set of axes.

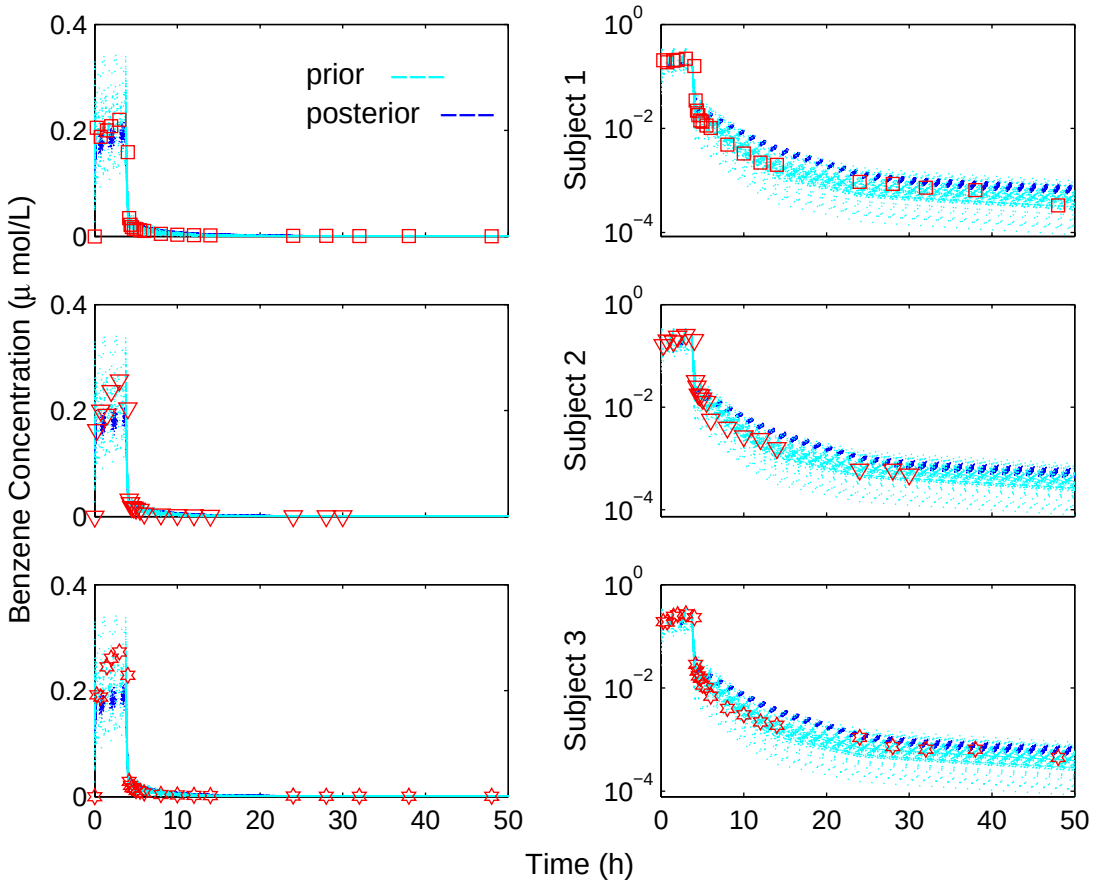

Figure 3: The model predictions versus data for benzene in exhaled air with higher exposure levels from Pekari, *et al*. (1992).

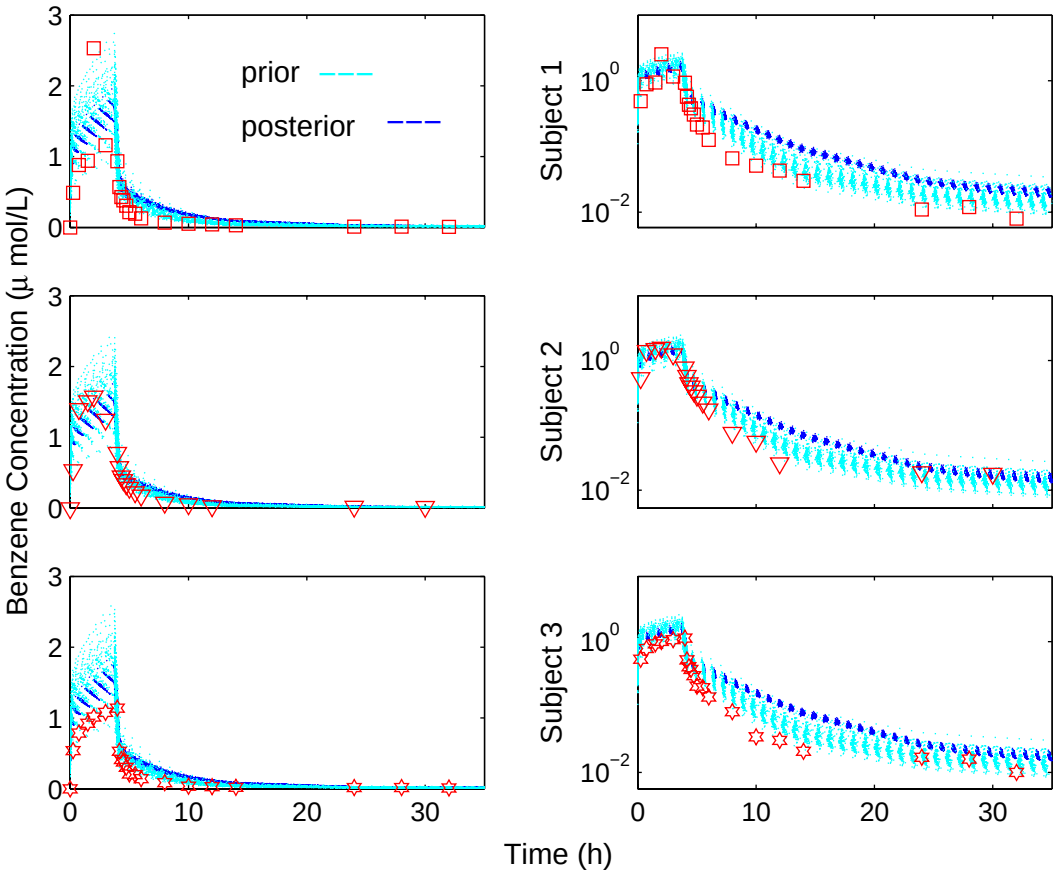

Figure 4: The model predictions versus data for benzene in blood with higher exposure levels from Pekari, *et al.* (1992).

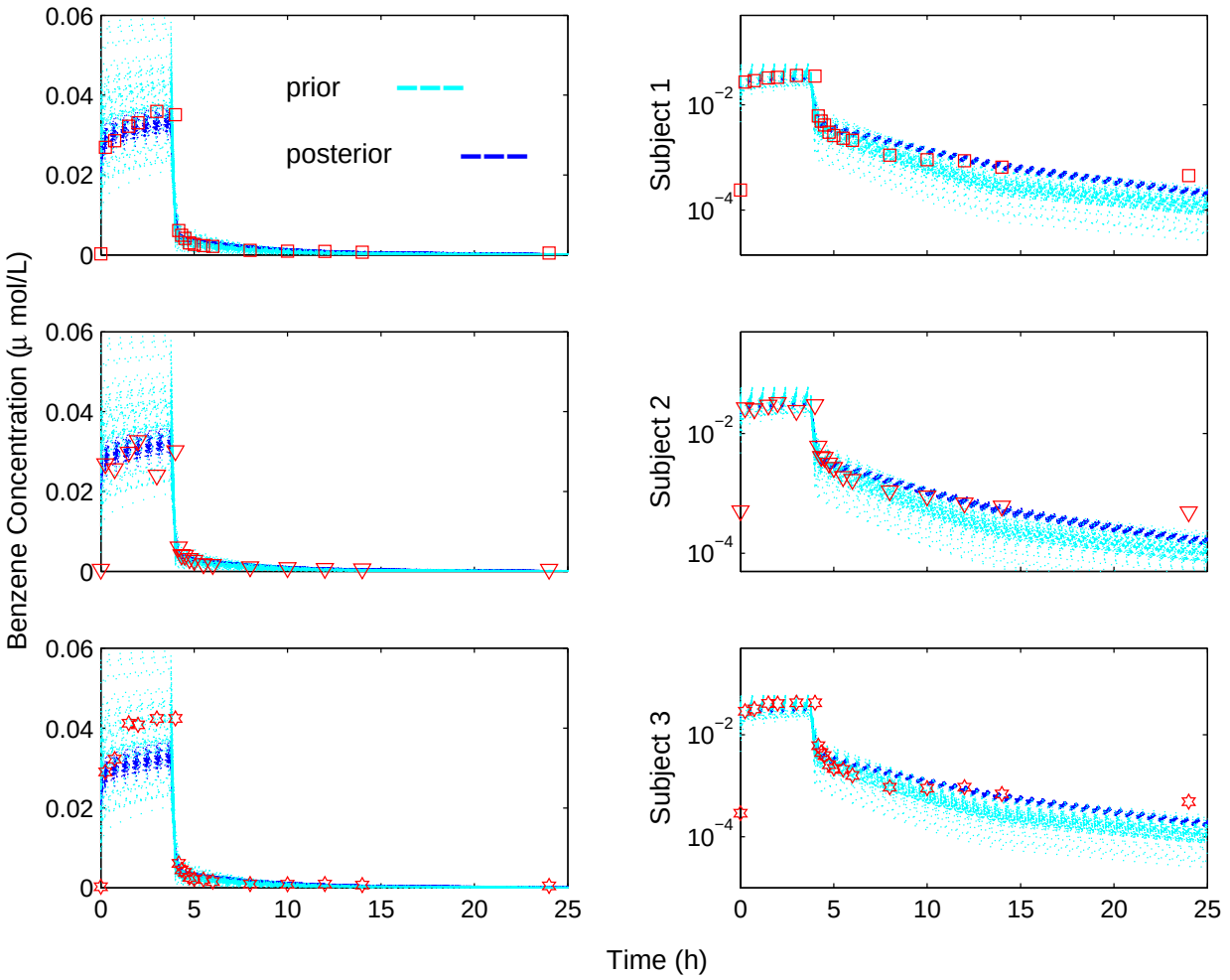

Figure 5: The model predictions versus data for benzene in exhaled air with lower exposure levels from Pekari, *et al*. (1992).

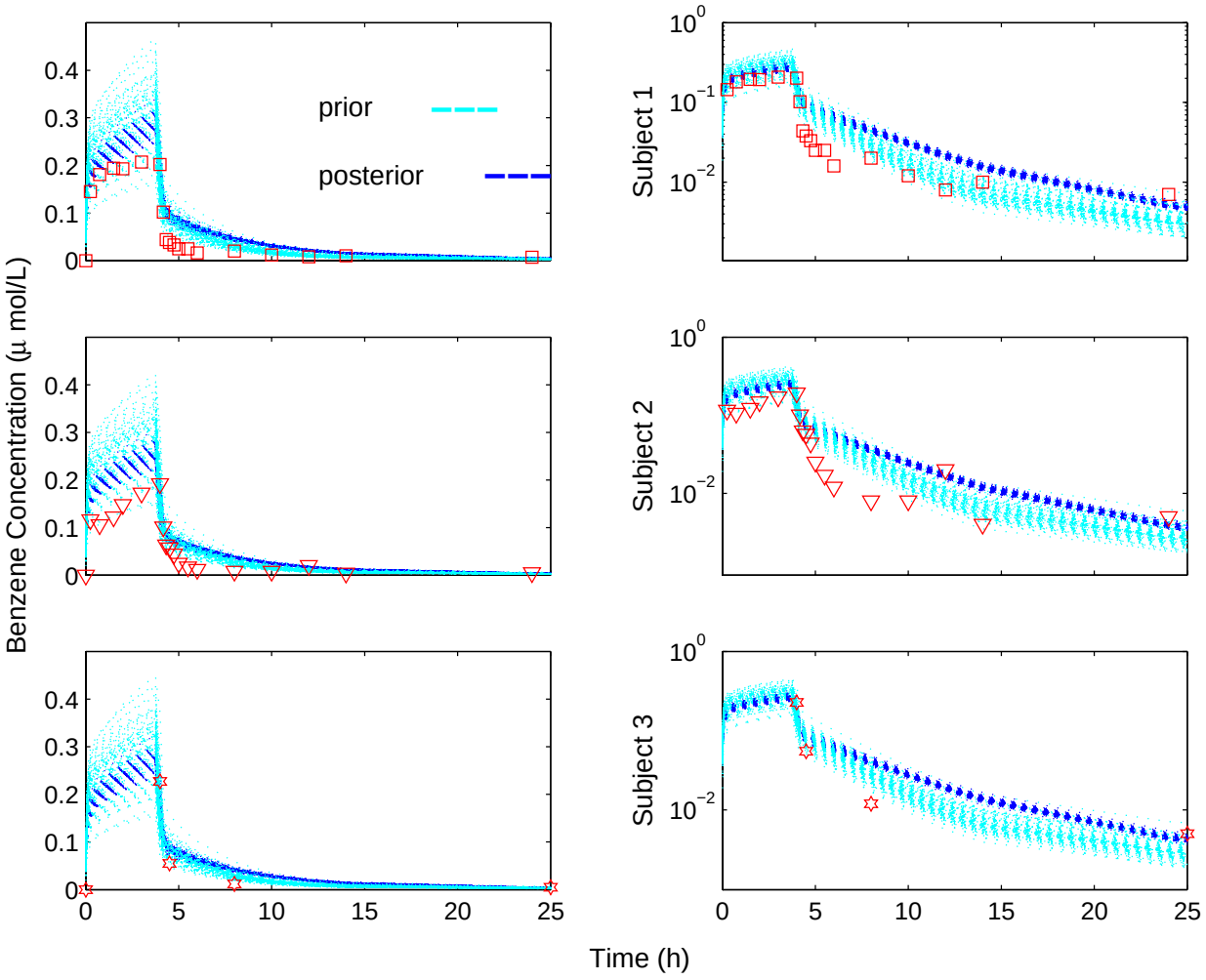

Figure 6: The model predictions versus data for benzene in blood with lower exposure levels from Pekari, *et al.* (1992).

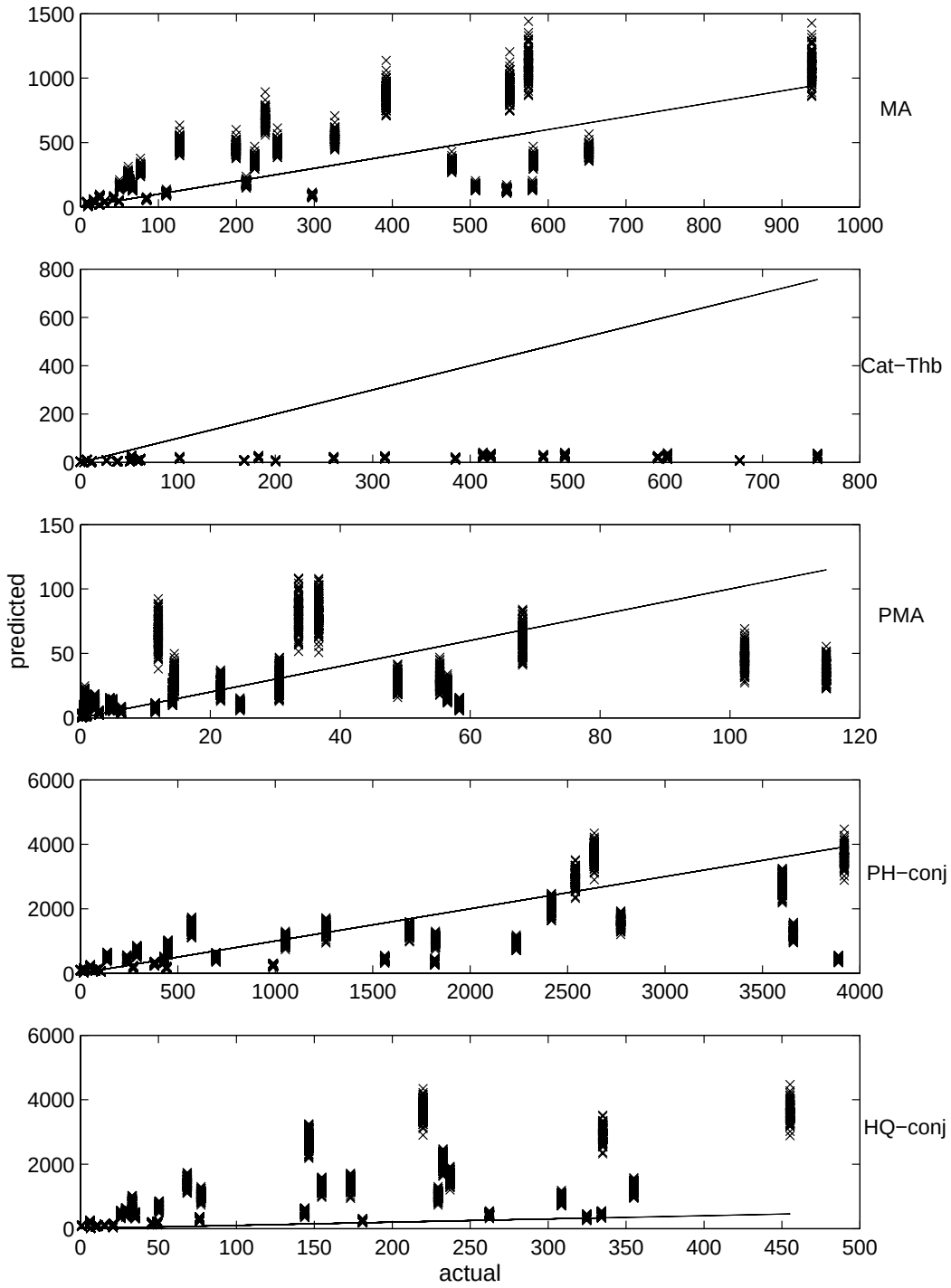

Figure 7: The model predictions versus metabolite data from Waidyanatha *et al.* (2004). The five urinary metabolites or metabolite groups simulated are: muconic acid (MA), catechol and trihydroxy benzene (Cat-Thb), phenol and phenol conjugates (PH), phenylmercapturic acid (PMA), and hydroquinone and hydroquinone conjugates (HQ).