BAYESIAN ANALYSIS OF PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL AND EXPOSURE RECONSTRUCTION FOR PERCHLOROETHYLENE

by

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(Under the Direction of JAMES V. BRUCKNER)

ABSTRACT

Perchloroethylene (PCE) is a pollutant distributed widely in the environment and the primary chemical used in dry cleaning. Liver cancer induced by PCE has been observed in mice, and central nervous system (CNS) effects have been observed in dry-cleaning workers. The objectives of this study were to 1) derive population distributions of physiologically based pharmacokinetic (PBPK) model parameters, which will subsequently be used in PCE exposure reconstruction, 2) predict the trend of percentages of PCE metabolized in the liver under different exposure conditions and 95th upper percentile for fraction PCE metabolized at a concentration of 1ppm with posteriors, 3) determine relationship between brain concentration of PCE and effect on visual evoked potentials, 4) perform sensitivity analysis of PBPK model parameters of PBPK model for PCE to model outputs to identify sensitive parameters to outputs and to ascertain effects of parameter transformation on sensitivity analysis results, and 5) reconstruct occupational exposure profiles to PCE with PBPK model based on sparse biomonitoring data. The 95th percentile for fraction PCE metabolized at a concentration of 1ppm was estimated to be 1.89%. Ventilation perfusion ratio (VPR) and blood/air partition coefficients (PB) in either original or transformed form were shown to be sensitive to variability in blood and alveolar air concentrations of PCE; variability in parameters clearance (ClC) in either form is most sensitive to model-predicted blood concentrations of trichloroacetic acid (TCA) or urinary

excretion of TCA. Atmospheric PCE levels in the working environment and background levels of PCE had high correlations with the biomarkers, blood and alveolar PCE concentrations. Lastly, posterior distributions of PBPK model parameters and exposure parameters were used to perform Monte Carlo (MC) simulations. Bootstrap sampling of MC sample with respect to likelihood of model outputs was used to construct distributions of exposure profiles.

INDEX WORDS: Perchloroethylene (PCE), Bayesian analysis, PBPK models, Markov chain Monte Carlo, Exposure reconstruction

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DEDICATION

This dissertation is a special gift for my family.

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ABBREVIATIONS

ATSDR	Agency for Toxic Substances and Disease Registry
AUC	Areas Under Curve
Cal	Alveolar Air Concentrations of PCE
CDC	U.S. Centers for Disease Control and Prevention
ClC	Clearance
CNS	Central Nervous System
CTCA	Blood Concentrations of TCA
CVen	Venous Blood Concentrations of PCE
CYP450s	Cytochrome P450 enzymes
DCA	Dichloroacetic Acid
ECFs	Exposure Conversion Factors
EPA	Environmental Protection Agency
FracK	Fraction of Kidney PCE Metabolism
FM03	Flavin-containing Monooxygenase 3
GI	Gastrointestinal
GSH	Glutathione
lnKm	Affinity constant in log form
MAE	Mean Absolute Value
MC	Monte Carlo
MCMC	Markov Chain Monte Carlo
MH	Metropolis-Hasting
MP	Mean of P value

ABBREVIATIONS

NAS	National Academy of Sciences
nAChRs	Nicotinic Acetylcholine Receptors
NCI	National Cancer Institute
NIOSH	National Institute of Occupational Safety and Health
NRC	National Research Council
NSCs	Normalized Sensitivity Coefficients
NTP	National Toxicology Program
PB	Blood/Air Partition Coefficients
PBPK	Physiologically Based Pharmacokinetic
PCE	Perchloroethylene
PFat	Fat/Blood Partition Coefficients
PLiv	Liver/Blood Partition Coefficients
PPARα	Proxisomal Proliferation Activation Receptor
PPC	Posterior Predictive Check
PRap	Rapidly Perfused Tissue/Blood Coefficients
PSlw	Slowly Perfused Tissue/Blood Coefficients
QPC	Pulmonary Ventilation Rate
SDP	Standard deviation of P values
SDs	Standard Deviations
TCA	Trichloroacetic Acid
TCE	trichloroethylene
TCVC	*S*-(1,2,2-trichlorovinyl)-L-cysteine

ABBREVIATIONS

TCVG	*S*-(1,2,2-trichlorovinyl)GSH
TRI	1,1,1-trichloroethane
TWA	Time-weighted Air
Urn	Urinary Elimination of TCA
VEPs	Visual Evoked Potentials
VOCs	Volatile Chemicals
VPR	Ventilation Perfusion Ratio
VSCCs	Voltage-sensitive Calcium Channels

CHAPTER 1

INTRODUCTION

Perchloroethylene (PCE) , or tetrachloroethylene, has been used widely in the dry cleaning, textile processing and metal degreasing industries (ATSDR, 1997). People using PCE in industries and dry cleaners are potentially exposed to PCE via inhalation. The general population may be exposed to PCE through contaminated food, air, water and consumer products (ATSDR, 1997). Maximum PCE levels detected in buildings with dry cleaners in New York city were 5,000 ug/m^3 (McDermott et al., 2005). Liver and kidney are the two major target organs of PCE toxicity; central nervous system (CNS) related toxic effects such as headache, dizziness and deficit in vision functions have also been reported (EPA, 1985). An association between CNS related effects and occupational or residential exposure to PCE has been observed in previous studies (Altmann et al., 1995; Schreiber et al., 2002).

There were two main goals of this research. The first was to conduct Bayesian analysis of a physiologically based pharmacokinetic (PBPK) model to derive population distributions of model parameters based on pharmacokinetic data and predict dosimetry related to liver cancer. The second was to reconstruct PCE exposure profiles for people working in dry cleaners based on biomonitoring data with the PBPK model developed in the previous step.

In this study, a PBPK model was integrated with a statistical population model. Markov chain Monte Carlo (MCMC) simulations were run with the population PBPK model. Posterior distributions of model parameters were derived based on MCMC simulation outputs. To reconstruct exposure profiles, Monte Carlo simulations were used to construct a matrix for exposure and PBPK model parameters by sampling from posterior distributions of the model parameters. Then likelihood of model outputs were calculated based on Monte Carlo simulation

outputs. Re-sampling with respect to likelihood was used to construct distributions of exposure profiles.

This dissertation includes a literature review of current knowledge of PCE (Chapter 2), followed by two chapters that discuss the background, methods, results of Bayesian analysis of a PBPK model for PCE (Chapter 3) and exposure reconstruction based on biomonitoring data (Chapter 3). The results of this research will benefit cancer risk assessment for PCE. The estimated distributions of fraction of PCE metabolized in liver is a key factor in calculation of PCE cancer risk.

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CHAPTER 2

LITERATURE REVIEW

The first objective of this research project is to derive a population physiologically based pharmacokinetic (PBPK) model, to improve estimates of liver and central nervous system (CNS) dose metrics, for use in deriving health-protective exposure guideline for PCE. The second objective of this research was to use a population PBPK model for PCE to reconstruct exposure levels in the environment from which biomonitoring data (i.e., blood and breath concentrations of PCE) were obtained. In this literature review, contamination, toxicity, biomarkers of exposure, pharmacokinetics, population PBPK model development and reverse dosimetry of PCE will be covered. The PBPK modeling and statistical methods used to conduct reverse dosimetry will be reviewed as well.

PCE contamination

PCE is a man-made chemical that is used primarily in dry cleaning, metal degreasing and chemical production and these industries consume 15% to 25% of total PCE consumption (EPA, 1985). PCE contamination is due mainly to release of PCE into ambient air by dry cleaners and industries using or producing PCE (ATSDR, 1997). PCE can easily volatize into air and is often found there. PCE can also be found in groundwater and surface water (ATSDR, 1997). PCE is also a contaminate common in drinking water (ATSDR, 1997). PCE can be found in some consumer products such as printing inks, adhesives, and lubricants (ATSDR, 1997). PCE contamination and exposure pathways are displayed in Fig. 2.1.

PCE toxicity

The toxicity of PCE is correlated highly to route of exposure and dose. The toxic effects are very diverse including neurological, carcinogenic, reproductive, developmental, genotoxic, immunological, systemic effects and even death (ATSDR, 1997). Since CNS effects are often observed in humans and the liver is a major target organ in mice and possibly humans, neurological and carcinogenic effects (especially liver cancer) will be addressed in detail.

Types of neurological effects are dependent on levels of PCE exposure. At extremely high exposure levels (> 1000 ppm), neurological effects can be observed. The primary concern is with possible effects in humans under exposure conditions like those in occupational and environmental settings. One controlled study by Altmann et al.(1990) revealed that there was a significant increase ($p < 0.005$) in visual-evoked potentials in persons exposed to 50 ppm PCE versus people exposed to 10 ppm PCE for 4 hours per day over 4 consecutive days. In a subsequent study (Altmann et al., 1992), the results of the previous study were confirmed and significant deficits in performance of vigilance and eye-hand coordination at 50 ppm were detected. For uncontrolled studies of dry cleaning workers exposed long term to PCE at time-weighted air (TWA) concentrations of 10-60 ppm, memory loss, concentration impairment, dizziness, impaired perceptual and intellectual function were observed (Lauwerys et al., 1983; Gregersen, 1988; Seeber, 1989; Cai et al., 1991; Echeverria et al., 1995). One study of people living above or next to dry cleaning facilities (Altmann at al., 1995) found there were increases in both response time in a continuous performance test and simple reaction time, compared to controls adjusted for age, gender and education.

Studies of animals exposed to PCE via inhalation and gavage (NTP, 1986 and NCI, 1977) revealed that mice but not rats developed hepatocellular neoplasms. The species difference may be due to different metabolic rates in mice and rats. Mice produce higher level of trichloroacetic acid (TCA) than rats (Odum et al., 1988). TCA is a major metabolite of PCE and is believed to induce peroxisomal proliferation in mouse liver (Goldsworthy and Popp, 1987). TCA can activate the proxisomal proliferation activation receptor α (PPARα), a nuclear receptor, resulting in an increase in proximal enzymes and CYP450s involved in lipid metabolism (Lash and Parker, 2001). Then proxisome proliferation generates reactive oxidative metabolites that can modify signaling pathway, cause cell death and reparative hyperplasia, and induce somatic mutation (Lash and Parker, 2001). Plasma protein binding is another key factor responsible for target tissue exposure and species-specific susceptibility of animals to TCA induced cancer. Binding capacities for TCA are different among humans (708), Rats (283) and mice (29) (Lumpkin et al., 2003). Greater binding capacities for TCA in humans means lower proportion of TCA available to liver and other tissues and longer residence time of this compound in the blood.

In a recent study by Lash et al. (2007), injury of isolated hepatocytes after exposure to PCE was reduced by CYP450 inhibition. Dichloroacetic acid (DCA), a minor metabolite of PCE, can also induce hepatocellular tumors in mice (Herren-Freund et al., 1987; Pereira, 1996). In a case-control study of humans, an increased risk of primary liver cancer was reported in male dry-cleaners. Alcohol consumption and smoking were accounted for (Stemhagen et al., 1983). However, the types of solvents and levels of exposure were not indicated; other solvents may be confounded this study. A small

increase of liver cancer risk was also observed among female dry cleaners (Lynge and Thygesen, 1990). Increased cancer risks of other types of cancer such as esophageal cancer (Ruder et al., 1994), cervical cancer (Anttila et al., 1995) and non-Hodgkin's lymphoma (Anttila et al., 1995) have also been observed. However, a number of epidemiological studies of occupationally-exposed groups have not revealed increased cancer risks in humans (Blair et al., 2003; Lynge et al., 2006; Mundt et al., 2003). A possible reason is that the results may be confounded by other covariates such as co-exposure to additional chemicals, alcohol consumption and smoking, socioeconomic status, sex and age etc.

Biomarkers of exposure

Biomarkers have been classified into three types: biomarkers of exposure, biomarkers of effects, and biomarkers of susceptibility (NAS/NRC, 1989). Since we use biomonitoring data to reconstruct concentration time profiles, our current focus is on biomarkers of exposure. Such biomarkers are generally concentrations of the parent compound or metabolite measured in body fluid or excreta. Use of biomarkers of exposure to reconstruct concentration time profiles may be difficult due to confounding factors such as co-exposure to other chemicals, which produce the same metabolites. The general biomarkers of exposure for PCE are blood or alveolar air concentrations of PCE or blood and urine concentrations of TCA.

The preferred biomarker of exposure to PCE is alveolar air or exhaled breath concentrations of PCE, since measurement of alveolar or exhaled air concentrations of PCE is simple, accurate and noninvasive. Alveolar air or exhaled breath concentration is a good biomarker for occupational and environmental exposure. In the occupational

exposure studies, there are significant correlations between time-weighted air concentration of PCE and exhaled breath concentrations of PCE measured immediately after a work shift ends. Correlation coefficients determined in these studies are 0.75 (Aggazzotti et al., 1994; Sole et al., 1990), 0.89 (Petreas et al., 1992) and 0.93 (Monster et al., 1983). Therefore, breath air concentrations of PCE after exposure can be reliable biomarkers of previous exposures. Thus, exhaled breath or alveolar air concentrations of PCE can be good indices of body burdens (Monster et al., 1979).

Blood concentrations of PCE are also good biomarkers of PCE exposure. There was a strong correlation between breath or alveolar air concentrations and blood concentrations of PCE (Petreas et al., 1992). Blood concentrations of PCE after a shift on Friday are correlated highly with the weekly TWA (Monster et al., 1983). However, limitations of this method are that it is invasive and expensive in terms of sampling and analysis.

Although the correlation between urinary TCA levels after a shift on Friday and the weekly TWA is good as indicated in a study by Monster et al. (1983), this biomarker may not always be reliable. TCA is not a specific metabolite of PCE. In addition, metabolism of PCE to TCA may be saturated at high exposure levels. One needs to be careful when using the amount of TCA excreted in urine as a biomarker at high exposure levels.

Pharmacokinetics of PCE

Absorption of PCE into the human body occurs via two pathways primarily. One is through the lungs into the bloodstream during an inhalation exposure; the other is across membranes of the gastrointestinal (GI) tract after oral exposure (ATSDR, 1997).

Absorption of PCE via skin is not significant in both humans and animals (ATSDR, 1997). The absorption of PCE via lung is dependent on pulmonary ventilation rate, exposure concentration and duration. Pulmonary ventilation rate is related to activities of humans such as exercise and rest. The total uptake of PCE is more affected by body fat than by pulmonary ventilation rate (Monster et al., 1979).

Since PCE is lipophilic, it primarily distributes into fat tissue. As indicated in the study of Guberan and Fernanderz (1974), about 50% of the body burden of PCE is predicted in adipose tissue after exposure to PCE for 8 hours at 100 ppm. PCE is distributed not only to fat tissue but also to liver, lung, kidney and brain. Two human fatalities following inhalation of high levels of PCE provided tissues from which the distribution of PCE in humans could be examined. One fatal case showed the brain concentration (36 mg/100 g) to be more than 120 times the lung concentration (Lukaszewski, 1979). Another case indicated that the liver concentration of PCE was higher than that in lung, kidney and brain (Levine et al., 1981). In addition, animal studies have shown that PCE crosses the placenta and distributes in the fetus and amniotic fluid (Ghantous et al., 1980).

PCE is metabolized by two pathways: cytochrome P450 (CYP)-catalyzed oxidation and glutathione (GSH) conjugation. Dominate PCE metabolism pathway is summarized in Fig. 2.2. PCE-oxide is the initial metabolite which can be further biotransformed to different chemicals (Lash and Parker, 2001). PCE-oxide is primarily biotransformed to trichloroacetal chloride, which reacts with water to form TCA. For rodents and humans, TCA is a predominant metabolite in urine and also can be converted to DCA. PCE is metabolized poorly in the human body, as approximately 1% is

converted to TCA (Monster et al., 1979). Metabolism of PCE to TCA is a saturable process (NTP, 1986).

A small proportion of PCE is metabolized via GSH conjugation pathway. *S*-(1,2,2-trichlorovinyl)GSH (TCVG) is produced at initial metabolic step which is catalyzed by GSH transferase and occurs in the liver. TCVG is further converted into *S*-(1,2,2-trichlorovinyl)-L-cysteine (TCVC). TCVC is activated by β-Lyase in kidney and biotransformed into thioketenes (Lash and Parker, 2001). In the liver, TCVC can be detoxified by N-acetylation. Flavin-containing monooxygenase 3 (FM03) can catalyze the transformation of TCVC into TCVC sulfoxide which can rearrange to form 2,2-dichlorothioketene. Dichlorothioketene can form DCA. Therefore, DCA can be formed in either GSH conjugation or P450 pathway. Once the oxidation pathway is saturated, the extent of GSH conjugation of PCE increases. Metabolites of GSH conjugation pathway are believed to be responsible for nephrotoxicity (Lash et al., 2007).

PCE is more extensively metabolized to TCA in rats than in humans (Volkel et al., 1998). In addition, only traces of DCA was detected in urine of persons after inhalation of PCE at 40 ppm for 6 hours; relatively large amount of DCA was detected in rats after exposure to equivalent dose (Volkel et al., 1998). In the study by Volkel et al. (1998), urinary excretion of TCA and N-acetyl TCVC is higher in rats than in humans. Therefore, rat is more susceptible to nephrotoxicity induced by PCE than humans.

TCA is a stable metabolite of PCE and poorly metabolized (Yu et al., 2000). TCA in blood is eliminated much slower in humans (90 to 100 hours) than in mice (7 hours) (Muller et al., 1972). Although humans produce less TCA than mice, TCA tend to stay in human bodies for a longer time. Human plasma has 2-fold higher TCA binding capacity

than rat plasma (Templin et al., 1995). Relatively high binding capacity of human plasma for TCA are speculated to be related to its larger number of binding sites and its high concentrations of albumin (Lumpkin et al., 2003). Plasma protein binding of TCA can affect its distribution, elimination and metabolism. TCA is charged at physical PH. But TCA can cross cell membrane by a bidirectional monocarboxylate transporter (Poole and Halestrap, 1993). The concentration of TCA at target site (hepatocytes) are governed by the concentration of free TCA in plasma (Lumpkin et al., 2003). Total metabolism of PCE has been proposed as a dosimeter for hepatotoxcity, due to linear relationship between hepatotoxicity and the extent of PCE metabolized by Buben and O'Flaherty (1985). In light of this observation, the fraction of the internal dose of PCE metabolized to TCA appears to the most appropriate dose metric at present to use in PBPK modeling.

Population PBPK model for PCE

A PBPK model has been defined as a mathematical model for xenobiotic absorption, distribution, elimination and metabolism using physiological approach (Lutz et al., 1980). PBPK models are used to describe and predict the time-course of concentrations of chemicals and their metabolites in the circulatory system and target organs, by use of anatomical, physiological, and biochemical information combined with chemical kinetic principles and chemical properties (Wagner, 1981). Models are constructed by linking a series of anatomically-relevant tissue compartments. Each compartment receives the chemical via the arterial blood and loses chemical via the effluent venous blood. The movement of chemicals through the tissue compartments can be described as diffusion-limited or perfusion-limited (Wagner, 1981). Metabolic clearance in tissue compartments can be described by appropriate equations such as the Michaelis-Menten equation,

Equation 2.1

$$\frac{V_{max}[S]}{[S]+K_m},$$

Where V_{max} is the maximum metabolic rate, K_m is the affinity of an enzyme to a specific substrate and [S] is the concentration of the substrate. In order to simulate the kinetic behavior of a chemical and its metabolites reliably with PBPK models, accurate values for input parameters are essential. Some parameters values can be found in the published literature; others need to be measured or estimated. PBPK models are developed because they can be used to extrapolate between species, to derive internal dose and quantify metabolism in the liver (Gearhart et al., 1993), to predict concentrations of chemicals in target organs and to reconstruct exposure (Georgopoulos et al., 1994). PBPK models have been involved in risk assessment for some chemicals. But uncertainty imbedded in model structures and parameters are still controversial. Evaluation of the suitability of a PBPK model is a critical step for the model used in risk assessment (Clewell et al., 2005). Sensitivity and uncertainty analysis techniques have been used to evaluate PBPK models (Clewell et al., 1994; Clewell et al., 2005).

Over the past three decades, a series of PBPK models for PCE have been developed. The characteristics of these models were summarized by Clewell et al. (2005). Most of the PBPK models have five compartments: lung, liver, fat, slowly perfused tissue and rapidly perfused tissue. The first PBPK model describing the kinetics of TCA was developed by Gearhart et al. (1993). Clewell et al. (2005) modified Gearhart and co-worker's model (1993) by assuming PCE can be metabolized to TCA in the kidney, and the TCA is excreted into urine. This modified PBPK model (Clewell et al., 2005) has been used for PCE risk assessment (Convington et al., 2007). There are no PBPK models

describing the metabolism of PCE via GSH conjugation pathway which is related to kidney injury. Hence, to estimate kidney dose metrics is not possible with these models. Different kinetic data used to develop and validate PBPK models for PCE caused the differences between these models. The differences in the kinetic data reflect different exposure levels, exposure pathway, species used and dose metrics measured.

A population PBPK model is a combination of a PBPK model and a statistical population model. The basic principle behind population PBPK models is that same differential equations in PBPK models will be used to describe the concentration/time profile for each subject, but the parameters of PBPK models will have different values for each individual. The statistical population model is a hierarchical model which has two levels: a population level and a subject level. At the population level, population means and variances for parameters in the PBPK models are random variables and follow specific distributions; at subject level, parameters for each subject are randomly sampled from the distributions of parameters at population level. A Bayesian approach is used to develop the population PBPK model. The Bayesian statistical analysis combines two types of information (Bernardo and Smith, 1994; Gelman et al., 1995). One is prior information for each parameter in PBPK models from previous literature; the other is data obtained from experiments. Then the posterior distributions of parameters can be derived as the product of the likelihood of the data and prior probability of the parameters. They are consistent with both the data and the prior distributions. It is impossible to derive an analytical expression of posterior distribution, because of the nonlinear form of the PBPK model. This is a major impediment to application of Bayesian analysis of PBPK models. However, this difficulty has been overcome by Markov Chain Monte

Carlo (MCMC) methods. MCMC methods have been defined as a class of algorithms for sampling from probability distributions based on constructing a Markov chain that has the desired distribution as its equilibrium distribution (Gilks et al., 1996). The advantages of these methods are that they can provide samples of parameter values from posterior distributions, even without knowledge of analytical expression of the posterior distributions (Gelman et al., 1996; Gilks et al., 1996). MCMC methods make widespread use of Bayesian analysis possible. Until now, many MCMC methods have been developed. The differences between them are the ways in which Markov Chains are constructed. Gibbs sampling (Geman and Geman, 1984) and Metropolis-Hasting (MH) algorithms (Metropolis et al. 1953; Hastings, 1970) are the two most popular methods to construct the Markov Chain. In MH, a proposal value of one parameter is sampled from a proposed distribution. Then two densities corresponding to the proposed value and the original value are calculated. By comparing the two density values, the proposed value or original value will be accepted as the parameter value. MH algorithm is thought to be more efficient in dealing with Bayesian population PBPK models (Bernillon et al., 2000). Gibbs sampling is simpler than MH. Actually, it is a special case of MH. The most important point of Gibbs sampling is to only consider univariate conditional distribution. This distribution is defined when all of the random variables except one are fixed. Such a conditional distribution is easier to define than complex joint distribution. It is often in a simple form such as inverse gamma, normal and other common distributions. Therefore, one can generate random variables from sequential univariate conditional distributions. The posterior inferences are based on the converged chain. The convergence can be monitored by the method developed by Gelman and Rubin (1992).

Population PBPK models for PCE in humans have been developed in several previous studies (Bois at al., 1996; Covington et al., 2007; Gelman et. al., 1996). MCMC technique has been used to capture population characteristics and uncertainty in risk assessment (Bois at al., 1996; Covington et al., 2007; Gelman et. al., 1996). In the two previous studies (Bois at al., 1996; Gelman et. al., 1996), data used in the analysis was restricted to one study by Monster et al. (1979). In the study by Monster et al. (1979), PCE blood and breath concentrations were measured after inhalation of PCE at 72 ppm and 144 ppm for 4 hours. Further, liver dose metric and faction of PCE metabolized, was estimated at different PCE levels (50 ppm and 1ppb) via inhalation by Bois et al. (1979). The estimates for different exposure levels are dose-dependent. For PCE level at 50 ppm, 95% confidence interval for faction of PCE metabolized is from 0.25 to 4.1%; for PCE level at 1 ppb, 95% confidence interval faction of PCE metabolized is from 15 to 58%. In the most recent study (Covington et al., 2007), model structure was more complex due to the inclusion of a sub-model for TCA. Fraction of PCE metabolized was also estimated at 1 ppb via inhalation. The 95 % confidence interval for this estimate is from 0.00526 to 0.0207. In the MCMC analysis by Convington et al. (2007), three independent studies (Fernandez et al., 1976, Monster et al., 1979 and Volkel et al., 1998) were used. Concentration-time profile data of TCA (Volkel at al., 1998) was used in the analysis. Differences in model structures and data used cause different estimates of PCE faction metabolized. Although kinetic data on TCA was used in analysis by Convington et al. (2007), only grouped data were used in the analysis. Hence, interindividual variability cannot be captured.

PBPK models have been developed for different species such as mice, rats and humans (Clewell et al., 2005). Significant sources of variability of PBPK model parameters are from differences in metabolism and mode of action due to sex and species differences (Lash and Parker, 2001). To reduce variability of PBPK model parameters, age-, sex- and species-dependent parameters can be introduced into PBPK model. Lash and Parker (2001) suggested to involve GSH conjugation metabolism pathway to improve estimates of metabolism, especially at higher doses of PCE.

Reverse dosimetry of PCE

Reverse dosimetry is also called exposure reconstruction. In the past decades, efforts were focusing on developing methods to reconstruct exposure profiles based on biomonitoring data. Almost all of these methods (Clewell et. al., 1999; Georgopoulos et al., 1994; Liao et al., 2007; Tan et al., 2006; Tan et al., 2007) rely on Monte Carlo simulations to synthesize data to reconstruct exposure profiles. One method by Georgopoulos et al. (1994) is to use an optimization approach to search space of exposure concentrations of PCE and find the best agreement between predictions obtained via simulations with a PBPK model and observations. Another method is to use Monte Carlo simulations to obtain distributions of exposure concentrations of chemicals (Tan et al., 2006). Before obtaining distributions of exposure concentrations, a distribution of reverse conversion factors is derived by utilizing the inverse of the biomonitoring variables by assuming a linear relationship between the assumed exposure conditions and model predictions. This method has been applied to trihalomethanes and other volatile chemicals (VOCs) (Tan et al., 2007; Liao et al., 2007). Limitations of both methods (Georgopoulos et al., 1994; Tan et al., 2006) are that they reconstruct distributions of

exposure levels, but do not provide information about exposure frequency and interval. A method proposed by Sohn et al. (2004) is based on Bayesian inference to reconstruct population-scale exposures. Sohn et al. (2004) suggested Monte Carlo random sampling and Latin Hyercube sampling instead of Gibbs samplimg.

All the methods described above are PBPK-model-dependent. Sometimes there is no enough exposure information available. Distributions of exposure parameters need to be derived based on reasonable assumptions. Uncertainty in exposure reconstruction will increase due to uncertainty from assumptions. Dowell et al. (1997) proposed a model independent method called artificial neural net work which has been used to develop in vitro and in vivo correlations. This promising method is useful when exposure data are not enough for exposure reconstruction.

Conclusions

The PBPK model for PCE developed and validated by Clewell et al. (2005) is a suitable model for estimation of dose metrics of metabolites. Convington at al. (2007) has applied this model to estimate fraction of PCE metabolized. Consider CNS effects, this model can be modified by adding a brain compartment. Instead of grouped data, individual data can be used in the analysis to capture interindividual variability and improve the estimates of faction of PCE metabolized. Chapter 3 describes Bayesian analysis of PBPK model for PCE in humans. Since previous studies (Georgopoulos et al., 1994; Liao et al., 2007; Tan et al., 2006; Tan et al., 2007) rely on Monte Carlo simulations to generate data, this technique can be used in our study to reconstruct exposure profiles. Chapter 4 presents using a PBPK model for PCE to reconstruct exposure profiles. Chapter 5 contains conclusions of this study and future work.

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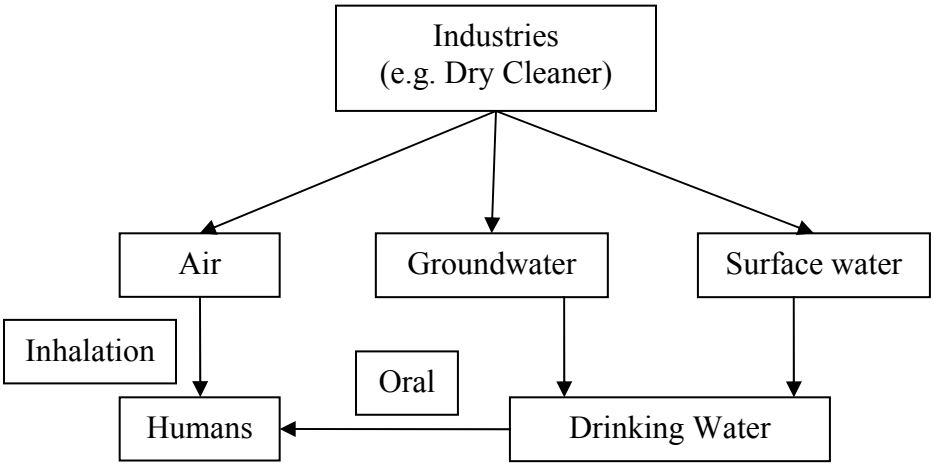

Figure 2.1. PCE contamination and exposure pathways.

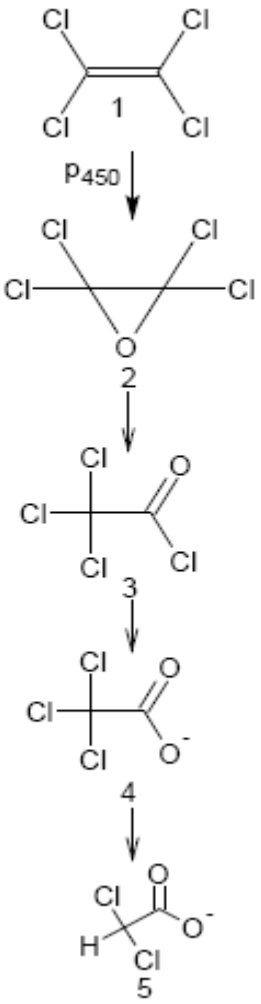

Figure 2.2. Dominant metabolism pathway of PCE. Enzymes: P450: Cytochrome P450. Metabolites: 1, PCE; 2, PCE epoxide 3, trichloroacetyl chloride; 4, trichloroacetate; 5, dichloroacetate.

CHAPTER 3

BAYESIAN ANALYSIS OF PYSIOLOGICALLY BASED PHARMACOKINETIC MODELING OF PERCHLOROETHYLENE IN HUMANS

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Abstract

Perchloroethylene (PCE) is a pollutant distributed widely in the environment and the primary chemical used in dry cleaning. Liver cancer induced by PCE has been observed in mice, and central nervous system (CNS) effects have been observed in dry-cleaning workers. A human physiologically based pharmacokinetic (PBPK) model was used to predict target tissue doses of PCE and its key metabolite, trichloroacetic acid (TCA) during and after inhalation exposures. A Bayesian approach, using Markov chain Monte Carlo (MCMC) analysis, was employed to combine information from prior distributions of model parameters and experimental data. Experimental data were obtained from five different human pharmacokinetic studies of PCE (Chiu, et al., 2007; Chien, 1997; Fernandez et al., 1976; Monster, et al., 1979; Volkel et al., 1998). The data include alveolar or exhaled breath concentrations of PCE, blood concentrations of PCE and TCA, and urinary excretion of TCA. Posterior analysis was performed to determine whether convergence criteria for each parameter were satisfied and whether the model with posterior distributions can be used to make more accurate prediction of human kinetic data. With posteriors, the trend of percentages of PCE metabolized in the liver was predicted under different exposure conditions. The 95th percentile for fraction PCE metabolized at a concentration of 1 ppm was estimated to be 1.89%. The estimation of population distributions of PBPK model parameters in this study will subsequently be used to support PCE exposure reconstruction.

Key words: Perchloroethylene (PCE), Bayesian Analysis, PBPK Models, Markov chain Monte Carlo

Introduction

Perchloroethylene (PCE) , or tetrachloroethylene has been widely used in dry cleaning, textile processing and metal degreasing industries. Workers employed in these establishments are likely to be exposed to PCE, mainly via inhalation. PCE can frequently be found not only in occupational settings but in living environments such as home, school and other locations (ATSDR, 1997). Although concentrations of PCE in indoor air are relatively low compared to those in occupational environments, potential effects of exposure to PCE in living environments should be considered due to longer exposure times. PCE is one of the most common contaminants of drinking water in the U.S. (Moran et al., 2007). Ingestion and inhalation during home use activities contributes to frequent findings of PCE in the general population (Blount at al., 2006).

A number of adverse health effects in humans including hepatorenal dysfunction, neurological deficits and certain cancers (e.g., liver, kidney) have tentatively been attributed to PCE and its metabolites (ATSDR, 1997). PCE has occasionally been linked to increased risks of cancers of the liver and other organs of dry cleaners (Stemhagen et al., 1983) and persons drinking contaminated water (Lee et al., 2005), but most epidemiology studies of occupational-exposed group have failed to find a significant association with liver cancer (Blair et al., 2003; Lynge et al., 2006; Mundt et al., 2003). Liver cancer in mice is thought to be related primarily to PCE's oxidative metabolites, including trichloroacetic acid (TCA), the major metabolite of PCE (Lash and Parker, 2001). Another major concern of PCE exposure is neurotoxicity. Impairment of intellectual function has been found in dry-cleaning workers (Seeber, 1989). Neurophysiological and neurobehavioral effects of PCE have been reported in acutely-and chronically-exposed persons (Altmann et al., 1992, and 1995). Changes in visual evoked

potentials (VEPs) have been frequently used to evaluate functional deficits in visual function and presumably the central nervous system (CNS) by volatile organic chemicals (VOCs) and other compounds. A significant relationship between change in amplitude of VEPs and PBPK model-predicted momentary brain concentration of trichloroethylene (TCE) was found in rats (Boyes et al., 2005). The mechanism of neurological action of PCE is related to its inhibition of nicotinic acetylcholine receptors and voltage-sensitive calcium channels (Bushnell et al., 2005). PCE similarly inhibits human and rat nAChRs expressed in oocytes in a dose-dependent manner (Bale et al., 2005).

Physiologically based pharmacokinetics (PBPK) models have been used widely to predict target tissue doses of chemicals, as well as to extrapolate from high to low dose, from animals to humans and from one exposure route to another. There are many PBPK models for PCE available. Some have been evaluated for their ability to predict blood concentrations of PCE and TCA and urinary excretion of TCA (Clewell et al., 2005). A PBPK model (Gearhart et al., 1993) that described formation and elimination of TCA was selected by Convington et al. (2007) for simulation of blood PCE and TCA time-courses, as well as cumulative urinary excretion of TCA by humans. These dosimetry predictions were used to calculate a Public Health Goal for PCE.

In order to quantify the uncertainty and variability of predictions of PBPK models for PCE, a Bayesian approach has been employed in several previous studies (Bois et al., 1996; Covington et al., 2007; Gelman et. al., 1996). The PBPK model used by Bois et al. (1996) and Gelman et. al. (1996) did not describe the behavior of TCA. In addition, the human data used in these efforts were obtained from just one controlled study (Monster et al., 1979), in which the inhaled PCE concentrations were 72 and 144 ppm. In the most recent investigation, Covington et al. (2007) modified the PBPK model of Gearhart et al. (1993) and used it in conjunction with

data from three research papers (Fernandez et al., 1976; Monster et al., 1979; Volkel et al., 1998). The PCE concentrations of the kinetics studies ranged from 10 -150 ppm. Workers and the general public are frequently exposed to lower levels. The data of Monster et al. (1979) and Volkel et al. (1998) were grouped, so information on inter-individual variability was not available.

In the current project, the PBPK model used by Covington et al. (2007) was modified to include a brain compartment, in light of PCE's potential neurological effects. The human kinetic data employed were obtained from five studies (Chien, 1997; Chiu et al., 2007; Fernandez et al., 1976; Monster et al., 1979; Volkel et al., 1998). All of the data used in the analysis were from individuals. Inter-individual variability can thereby be captured and estimated more reliably. The inhaled PCE concentrations in two of the studies (Chien, 1997; Chiu et al., 2007) were less than 10 ppm. These exposure levels are very close to those dry cleaning workers encounter. Incorporation of these data into our analysis should make the posterior distributions of model parameters more reliable for estimating target tissue dosimetry for PCE risk assessment. The fraction of PCE metabolized in the liver has been proposed to be a reasonable dosimeter for liver toxicity (Buben and O'Flaherty, 1985). The fraction of PCE metabolized per liver or body weight has been estimated in previous studies with different PBPK models (Clewell et al., 2005). These estimations of the fraction of PCE metabolized in the liver assumed constant exposure. In real life, exposures to PCE over a day or work week are quite variable. It is important to be able to estimate the fraction of PCE metabolized by the liver under different exposure conditions and to assess trends of the estimates as exposure time and exposure level decrease.

The overall goal of this work is to obtain population distributions of PBPK model parameters, that will be used later for PCE exposure reconstruction. Furthermore, posterior

distributions obtained by MCMC analysis will be used to predict liver (e.g., percentage of PCE metabolized) and brain (concentration of parent compound) dosimeters for a 4-h inhalation exposure of 11 human subjects to 50 ppm PCE over 4 days.

The data and PBPK model structure used in this analysis will be described in detail, as will be the hierarchical Bayesian analysis process. Once posterior distributions of model parameters were obtained, a posterior predictive check was performed. Further, the use of posteriors to predict PCE metabolism in liver and brain concentrations of PCE was explained. Finally, sources of uncertainty imbedded in estimation of the fraction of PCE metabolized and PCE metabolism at low exposure levels and different exposure durations will be presented.

Methods

A hierarchical population model was integrated with the modified model for PCE, in order to predict the fraction of PCE metabolized in the liver under various exposure conditions typical for a general population; and assess the degree of correlation between CNS effects and PCE concentrations in the brain. Individual data from previous studies and prior distributions of population model parameters in the PCE model from previous analyses were used to obtain posterior distributions of model parameters with a Bayesian approach. The posteriors were used to predict the fraction of PCE metabolized in the liver under various conditions and the concentrations of PCE in the brain.

Relevant data

The data used in MCMC analysis were human blood and breath PCE concentrations, as well as blood concentrations and urinary excretion of TCA. The human data were obtained from four published studies (Chiu et al., 2007; Fernandez et al., 1976; Monster et al., 1979; Volkel et

al., 1998) and one unpublished study (Chien, 1997). In these investigations, human exposure levels were from 0.054 to 150 ppm. All of data sets used in this analysis are summarized in Table 3.1.

In Fernandez et al. (1976), post-exposure alveolar air concentrations of PCE were measured after 24 subjects were exposed to PCE at various vapor concentrations for different lengths of time. The amounts of TCA excreted in the urine were available for 2 subjects exposed to 150 ppm PCE for 8 hours and monitored at 4 post-exposure time-points. In Monster et al. (1979), blood concentrations and exhaled air concentrations of PCE were available for each of 6 male subjects exposed to PCE at 72 or 144 ppm for 4 hours via inhalation. In Volkel et al. (1998), blood concentrations of TCA were presented at only 2 time-points after 6 subjects were exposed to PCE at 10, 20 and 40 ppm for 6 hours via inhalation. Bayesian analysis was not conducted with just 2 time-points. The amount of TCA excreted in urine was measured at several time-points for each individual. These individual data were obtained from the authors by personal communication. In Chiu et al. (2006), alveolar air concentrations and blood concentrations of PCE were presented for each of 6 subjects during and following inhalation exposure to 1 ppm PCE. Blood concentrations and the amount of TCA excreted in urine for each of the 6 subjects were presented at several time-points both during and post exposure. In Chien (1997), alveolar air concentrations of PCE were recorded for only one subject after being exposed to different PCE vapor concentrations for various length of time. The exposure levels of PCE ranged from 0.054 to 4.935 ppm.

PBPK Model

The PBPK model utilized by Covington (2007) was modified by adding a brain compartment. In the current study, only the inhalation exposure pathway was considered. The schematic of the modified PBPK model is shown in Fig. 3.1.

In MCMC analysis of the PBPK model, it is assumed that the model parameters are independent. In reality, some parameters are correlated highly with one other, such as: cardiac output and alveolar ventilation; and maximum rate of metabolism and Michaelis-Menten constant. If correlations are ignored, much more time may be needed for the chain to converge. This problem is solved by defining a new parameter that can maintain correlation between the two parameters (Hack et al., 2006; Covington et al., 2007). In this analysis, ventilation perfusion ratio is defined as the ratio of alveolar ventilation and cardiac output, and scaled clearance is defined as the ratio of maximum rate of metabolism and the Michaelis-Menten constant.

Random sampling is employed in the MCMC analysis. This may break the mass balance among fractional blood flows and fractional tissue volumes. To avoid this, a fractional blood flow is obtained by summing fractional blood flows (unit one) minus the sum of fractional blood flows randomly sampled (Gelman et al., 1996[a]; Hack et al., 2006; Covington et al., 2007).

MCSim (Bois et al., 2002) is popular software used for MCMC analysis of PBPK models. The PBPK model used in the analysis needed to be transformed into the format recognized by MCSim. To assure the PBPK model was converted correctly, simulations under the different exposure conditions of data sets (Chiu et al., 2007; Fernandez et al., 1976; Monster et al., 1979; Volkel et al., 1998) were performed. Comparisons between simulated results and real data were made.

Markov chain Monte Carlo analysis

A hierarchical population model (Bois et al., 1996; Gelman et al., 1996[a]; Hack et al., 2006; Covington et al., 2007) was employed in the Bayesian approach. A population level and subject level were included in the hierarchical model. The structure of the hierarchical model was as shown in the Fig. 3.2. In the population level, a prior distribution for each PBPK model parameter was defined as a distribution with mean (μ) and variance (Σ^2). Prior distribution of the population mean was defined as a distribution with mean (M) and variance (S^2). For population variance (Σ^2), it follows a distribution with parameters (K).

At the subject level, prior distributions of PBPK model parameters were defined based on random sampling from the distributions with the population mean (μ) and variance (Σ^2). Model error can be partitioned into measurement error, misspecification of model structure, inter-individual variability and intra-individual variability among subjects (Bois et al., 1996). However, quantification of each part of model error was not considered here. The total error from each source was assumed to follow a log-normal distribution with mean zero and variance defined by a separate distribution (Bois, 2000; Bois et al., 1996; Gelman et al., 1996[a]; Hack et al., 2006; Covington et al., 2007).

The posterior distributions of the PBPK model parameters of Covington et al. (2007) were used as prior distributions. The prior distributions for brain physical parameters were taken from Chien (1997). In order to be certain the values of posterior distributions were in a biologically-reasonable range, truncated distributions are used. The upper and lower bounds of each truncated distribution are set to the mean plus or minus 2.5 times the standard deviation. Since some parameters cannot be negative, meaningful bounds are set separately.

In the previous studies (Bois, 2000; Bois et al., 1996; Hack et al., 2006; Covington et al., 2007) of the population variance (Σ^2), a prior distribution was defined as inverse gamma with parameters α and β , which is within the conditional conjugate family (Gelman, 2006). In this study, prior distribution of population variance (Σ^2) was still defined as inverse gamma distribution. As the components of model error are complex, there is almost no information on the variance of model error. As before, the prior distribution of the variance of model error was defined as log-normal (Bois, 2000; Bois et al., 1996; Gelman et al., 1996[a]; Hack et al., 2006; Covington et al., 2007). The values of prior distributions of PBPK model parameters are listed in Table 3.2-3.3. Three parallel chains were run with the same prior distributions and same data sets, but with different random seeds (initial values), which resulted in different starting values. Each chain was run 60,000 iterations.

The method of Gelman (1996[a]) was used to diagnose whether or not the chain converged. The potential scale reduction (R) was calculated for each parameter as the ratio of maximum and minimum of variances including within chain variance and between chain variance for each parameter. The critical value was set at 1.2. A value less than 1.2 indicates the chain converged (Gelman, 1996[a]). That means variance for one parameter can be reduced by most 20% if the iterations of the chain increase.

A set of values of model parameters drawn from posterior distributions were used to simulate new data with the PBPK model used in this analysis. Two statistics: area under the curve (AUC) of blood concentrations of PCE; and mean absolute error (MAE) were computed with new data and observed data for 100 times. The statistics computed with new data defined as T_s and the statistics computed with real data defined as T_o . Then record the number of times

when simulated statistics T_s are larger than observed statistics T_o to estimate the *p* value defined as in the posterior predictive check (PPC) method (Gelman et al., 1996[b]). That

Equation 3.1

$$p = 1 - \sum \frac{I(T_S \leq T_O)}{N}$$

here **I** is indicator function (a function equals to 1 if the condition in the bracket is true; otherwise, it equals to 0), and **N** is the times of statistics calculated. To obtain the distribution of *p* values, the above procedures need to be repeated 100 times. If model fit is perfect, the mean of p values (MP) is about 0.5; the standard deviation of p values (SDP) is around 0.289 (Yano et al., 2001). The acceptance region for the pair of mp and sdp is defined as

Equation 3.2

$$(MP - 0.5)^2 + SDP^2 \leq (0.5)^2$$

To estimate the distributions of the fraction of PCE metabolized in the liver under various exposure times and PCE exposure concentrations, Monte Carlo simulations were performed. Model parameter spaces were constructed from the last 5,000 parameter sets at population level of MCSim output for each of three chains. Here only inhalation exposure to PCE is considered. To capture the trend in the faction of PCE metabolized in liver under different conditions, the exposure time is set from 0.24 to 24 hours by 0.24 hours and concentration of PCE is set from 1 ppm to 100 ppm by 10 ppm. A total of 15,000 parameter sets were used to run PBPK model under various conditions, and the estimates of the fraction of PCE metabolized in the liver were

calculated based on simulation outputs, that is, the amount of PCE metabolized in liver divided by the amount of PCE inhaled.

Further, Monte Carlo simulations were run based on the parameters space constructed from the last 5,000 parameter sets at population level of MCSim output for each of three chains to determine the distribution for the fractional metabolism at low concentration 1 ppb via inhalation continuously.

PCE- induced CNS effects were observed in a study performed by Altmann et al. (1990). In this study, 22 healthy males were selected and randomly divided into two groups. Each group was exposed to PCE at 10 or 50 ppm for 4 hours daily and for 4 consecutive days. Blood concentration of PCE was sampled at 3 time-points: immediately before exposure (8:00 h), during exposure (10:00 h) and at the end of the exposure (12:00 h) for 6 days, including days 1 and 6 without exposure. VEPs were measured with respect to N75, P100 and N150 peaks after the first 2-hours of inhalation on day 1 –day 5. To explore the relationship between brain PCE concentration and CNS effects , the posterior population mean was used to perform Monte Carlo simulation under the same experimental condition as in the study performed by Altmann et al. (1990).

Results

Markov chain Monte Carlo analysis

As shown in Table 3.4, the potential scale reductions (R) of all parameters are less than 1.2. The convergence criteria are satisfied by all parameters in the PBPK model. The last 5,000 iterations can be used to estimate the posterior distributions.

The posterior mean distributions of all model parameters obtained via MCMC analysis are presented in Table 3.4. The posterior and prior variance distributions are summarized in Table 3.5. It is obvious that the variance of the population mean decreased for all parameters. For the parameters responsible for metabolism and elimination, population mean estimates were markedly affected except for the Michaelis-Menten constant in log form (lnKm) and the fraction of kidney PCE metabolism to TCA (FracK). The population mean estimates of the ventilation perfusion ratio (lnVPR) and pulmonary ventilation rate (QPC) are significantly different from priors ($p < 0.01$). For the parameters of blood flow into different compartments, except for fat and brain, the estimates of population mean have a big shift from the corresponding priors. The posteriors of the partition coefficients and volume of distribution of TCA did not change much from the priors in terms of both estimates and variance of population means.

Comparisons, between model simulations with priors of population means and simulations with posteriors of population means based on selected experiments, are shown in Figs. 3.3-3.7. In most cases, posteriors of population means give better fit of experimental data than priors do. Posterior means for each subject with maximum likelihood gave predictions that are even closer to experimental data than population posterior means. As shown in Fig. 3.3, predictions with population posteriors (dashed line) make a little improvement compared with those with priors (solid line); predictions with subject specific posteriors (dotted line) fit the kinetic data (asterisks) very well.

The last 1,000 samples of each parameter chain were used to calculate correlation coefficients for posterior means. The correlation coefficients were summarized in Table 3.6. Most of the absolute values of correlation coefficients were less than 0.1, which means correlation between two parameters is not significant. The absolute values of correlation

coefficients for fractions of blood flow to tissues are larger than 0.1 due to constrains on the sum of fractions of blood flows to tissues. Correlation of parameters and outputs such as amount of TCA excreted in urine were also calculated by Convington et al. (2007).

For each statistic, area under the curve (AUC) for PCE blood concentration and mean absolute error (MAE), the mean of p values (mp) and standard deviation of p values (sdp) were calculated and listed in Table 3.7. As shown in Fig. 3.8, two pairs of MP and SDP are located in the acceptance region. Therefore, posteriors obtained via MCMC process can be used to make predictions.

Monte Carlo simulations

The means of the fraction of PCE metabolized in liver, derived from the output of Monte Carlo simulations, are displayed in Fig. 3.9. The fraction of PCE metabolized in the liver decreases with increase in exposure time and exposure concentration of PCE. However, as exposure time and exposure concentrations of PCE decrease, the percentage of PCE metabolized in liver tends to stabilize at 1%. In the Bois et al. (1996) study, the estimated fraction of PCE metabolized in liver decrease as exposure levels increase. This trend agreed with our result.

Further, results of Monte Carlo simulations of continuous exposure to PCE at 1 ppb via inhalation based on posterior distributions of model parameters were summarized in Table 3.8. The median of fraction of PCE metabolized in liver via inhalation for continuous exposure to PCE at 1 ppb is 0.973% which is slightly lower than the predictions 1.02% by Convington et al. (2007) and 1.1% by Clewell et al. (2005). The upper 95^{th} percentile of PCE metabolized in liver was 1.89% which was slightly lower than 2.07% by Convington et al. (2007). The 95%

confidence interval of fraction of PCE metabolized in liver is (0.498-1.89%) which was substantially different from the estimated values (15-58%) by Bois et al. (1996).

Posterior population mean of model parameters were used to predict blood and brain concentrations of PCE for inhalation exposure to PCE at 50 ppm for 4 hours per day for 4 days (Altmann et al., 1990). As shown in Fig. 10, predicted blood concentrations of PCE (solid line) agree with observed venous blood concentrations of PCE (asterisks) ± one standard deviation (brackets). The predicted brain concentrations of PCE (dotted line) are higher than the predicted blood concentrations of PCE.

Discussion

An important aim of this project was to combine an appropriate PBPK model and MCMC methodology for Bayesian inference, in order to better predict the metabolism and toxicokinetics of PCE and its major metabolite, TCA. As described below, the posterior model parameter distributions generated by Covington et al. (2007) were adopted as the priors for the current exercise. Additional PCE and TCA kinetic data from individual study subjects and exposure levels were analyzed to derive posterior parameter estimates and ranges at the population level. Subject-level distributions of PBPK model parameters were obtained by random sampling from population mean and variance distributions. These values were then used in the PBPK model, along with experiment-specific exposure and physiological parameters, to generate simulations of PCE alveolar air and blood concentration versus time profiles, as well as cumulative urinary TCA excretion plots. It can be seen in Figs. 3.3 – 3.7 that the posterior-level model predictions were in somewhat better agreement with the empirical data than prior model predictions. This was particularly true for the simulations of TCA excretion. There was excellent agreement

between the experimental data and subject-specific model predictions. Thus, integration of a PBPK model into a statistically-rigorous framework that accounts for variability and errors can result in quite accurate simulations of PCE and TCA kinetics, even with low-level PCE exposures.

In the present study, the prior distributions of population variance are defined as inverse gamma distribution. However, posterior distributions will be sensitive to parameter values of gamma distribution, if small values of population variance ($\Sigma 2$) are possible for some datasets (Gelman, 2006). A half-Cauchy distribution is not defined in MCSim 5.0. In the future, more choices of prior distributions should be defined in MCSim, especially half-Cauchy.

PCE and most other lipophilic VOCs partition into neuronal lipids and thereby inhibit a variety of CNS functions. According to a commonly-accepted mode of action, the VOCs impair propagation and regeneration of membrane action potentials by their mere physical presence in axonal membranes (Bruckner et al., 2008). Thus the higher the target organ/cell dose (i.e., the higher the neuronal concentration) of VOC, the greater the magnitude of dysfunction. As noted in the Introduction, Boyes et al. (2005) found a statistically-significant relationship between brain trichloroethylene concentration and reduction in amplitude of VEPs in rats. Levels of 1,1,1-trichloroethane (TRI) measured in the blood and brain increased in direct proportion to one another in mice inhaling the VOCs (Warren et al., 2000). Both blood and brain TRI levels during exposures were strongly correlated with inhibition of locomotor activity. In the current project, the PBPK model was run to simulate human blood and brain PCE levels under the exposure conditions described by Altmann et al. (1990). The simulated blood and brain levels largely mirrored one another throughout the repetitive 4-day inhalation regimen. The brain PCE concentrations did exceed the blood concentrations during the last 2 hours of each day's

exposure (Fig. 3.10). There was a relatively linear relationship between VEP alteration and predicted brain PCE concentration in most of the range of concentrations. These findings and those of the other research groups support a "nonspecific" mode of action.

Recent studies of modes of action of inhaled anesthetics and other VOCs have revealed the existence of multiple molecular mechanisms (Hemmings et al., 2005). In vitro studies of rat and human recombinant receptors expressed in Xenopus oocytes have identified several neuronal membrane targets of PCE, including nAChRs, VSCCs and N-methyl-D-aspartate receptors (Bale et al., 2005; Bushnell et al., 2005; Raines et al., 2004). Nevertheless, the target organ/cell dose of parent compound will still determine the magnitude of effect at these sites. Although brain PCE time-course data have been obtained in rodent studies, there are no such human data with which to verify the accuracy of our PBPK model predictions. Therefore, there is uncertainty imbedded in the model's input parameters, particularly those related to PCE disposition in the brain. Nevertheless, our model should be useful in simulating PCE concentrations in the human brain under exposure conditions linked with CNS dysfunction.

It is widely recognized that PCE must undergo biotransformation to biologically-active products, in order to exert cytotoxicity, mutagenicity and/or carcinogenicity (ATSDR, 1997; Buben and O'Flaherty, 1985, Lash and Parker, 2001). PCE is metabolically activated by two pathways: cytochrome P450 (CYP450)-catalyzed oxidation; and glutathione S-transferase (GST)-catalyzed glutathione (GSH) conjugation. Oxidation of PCE by CYP2B6 in humans produces PCE-oxide, a relatively stable epoxide that contributes to cytotoxic effects in the liver. This metabolic intermediate is converted to several products, the primary of which is the reactive trichloroacetyl chloride (Lash and Parker, 2001). It in turn reacts with water to form TCA, the major urinary metabolite in rodents and humans (Volkel et al., 1998). Some TCA can be

dechlorinated to form dichloroacetic acid (DCA). DCA and TCA, in sufficiently high concentrations, can affect cell signaling proteins by altering expression of genes responsible for hepatocellular growth, inhibition and apoptosis (Bull, 2000). TCA is more potent than DCA in causing peroxisome proliferation, which in turn generates reactive oxygen moieties that produce oxidative stress and 8-hydroxydeoxyguanosine DNA adducts (Torasson et al., 1999). A small proportion of PCE undergoes GSH conjugation, but the cytotoxic, mutagenic metabolites that are formed via this pathway primarily affect the kidneys (Lash and Parker, 2001). Focus in the current manuscript was limited to liver injury and cancer. It is likely that a number of PCE's metabolites act in concert to damage hepatocytes and cause neoplasia in mice. Several of these metabolites are quite unstable and therefore difficult to quantify. TCA can be easily measured in blood, tissues and urine. Total metabolism of PCE has been proposed as a dosimeter for liver cancer, due to the complexity of the processes by which multiple metabolites may act. In light of these problems, the fraction of the internal dose of PCE metabolized appears to the most appropriate dose metric at present to use in PBPK modeling.

The current Bayesian assessment, coupled with MCMC analysis, resulted in predictions that a small fraction (~ 1%) of low doses of PCE will be metabolized by the liver. Bois et al. (1996) were among the first to use a PBPK model and Bayesian inference to model statistical distributions of proportions of systemic doses of PCE metabolized by humans. These scientists calculated the fraction metabolized at a constant 50-ppm vapor exposure to range from just 0.52 – 4.1% (95% confidence intervals). In contrast, the computed fraction ranged from 15 – 58% for inhalation of 1 ppb. It should be recognized that no PCE metabolite data were available to Bois et al. (1996), so it was necessary for them to rely upon concentrations of the parent compound measured by Fernandez et al. (1976) in experimental subjects' blood and breath. The model

used by Bois and co-workers did not include a submodel for TCA. Clewell et al. (2005) estimated that much smaller fractions of low doses were metabolized (i.e., 1.1% of 1 ppb continuously inhaled and 2.6% of 1 μg/kg ingested daily). In this instance, model parameters were estimated from a visual fit of the human urinary TCA excretion data of Volkel et al. (1998). Covington et al. (2007) subsequently utilized human data from 3 published studies (including Volkel et al.), as well as MCMC analysis to recalibrate the biotransformation parameters. Predictions of the median fractions of PCE metabolized, at the 1 ppb and 1 μg/kg/day exposure levels, were only slightly lower than previously reported by Clewell et al. (2005) (i.e., 1.0 vs. 1.1% and 2.5 vs. 2.6%). Chiu et al. (2007) recently estimated apparent metabolic clearance to be 11.5 – 15.2% in 7 men inhaling 1 ppm PCE for 6 h. The researchers acknowledged substantial variability and uncertainty in their empirical estimates. The presently computed metabolic clearance value of ≤ 1% supports the values of Clewell et al. (2005) and Covington et al. (2007).

It is not surprising that our central estimates of hepatic PCE metabolism are substantially different from those of Bois et al. (1996), but agree with estimations by Covington et al. (2007). The present PBPK model for PCE and its metabolite TCA is substantially the same as that employed by Covington and her colleagues, except for the addition of a brain compartment. As discussed by Clewell et al. (2005), estimation of metabolic parameters and related indices is not reliable and can be problematic when only kinetic data for the parent compound are utilized, as was the case in Bois et al. (1996). However, the current Bayesian approach and MCMC analysis represents a significant expansion and more detailed assessment than that by Covington et al. (2007). They had to use grouped toxicokinetic data, including summarized values of Dekant et al. (1986), for estimating the fractional amount of PCE converted to TCA in the liver. The prior distributions of model parameters we used are the posteriors derived by Covington et al. (2007).

It was possible to obtain individual subject data from Dekant, as well as additional low exposure-level data from Chien (1997) and Chiu et al. (2007) to work with in the current effort. As a result, the uncertainty in the fraction of PCE metabolized associated with inter-individual variability could be estimated with greater confidence. In addition, the inclusion in this study of the very low-concentration (0.054 – 4.94 ppm) inhalation data of Chien (1997) should aid in reducing parameter uncertainty and enhancing model predictability for low-level occupational and environmental exposure scenarios. With the improvement of analytical sensitivity, additional kinetic data from low-dose experiments may become available for further refinement of predictions of PCE biotransformation at environmental concentrations.

The estimates of both Bois et al. (1996) and Covington et al. (2007) of the fraction of PCE metabolized in the human liver are based on the assumption of constant PCE intake. Different exposure conditions are considered in the present investigation. As exposure durations become longer (0.24 to 24 hours) and inhaled vapor concentrations rise (1 to 100 ppm), the fraction the systemically-absorbed dose of PCE metabolized by the liver diminishes. Conversely, as exposure duration and level decrease, the fraction of the dose of PCE metabolized increases. We found that this fraction is stable at ~ 1% for low doses. Adipose tissue, the major storage site in the body for PCE and other lipophilic chemicals, may contribute to this stabilization phenomenon. Fat serves as a depot, from which PCE is slowly metered back into the systemic circulation, such that its fractional metabolism remains unsaturated and around 1%, unless moderate to high exposures occur.

The principal issues in PCE cancer risk assessment are the intrinsic uncertainty and variability in the fraction metabolized (i.e., metabolically activated) at low inhalation and drinking water concentrations. Values as high as 58 and 79% have been adopted by regulatory

agencies in the U.S. estimate cancer risks for humans in occupational and environmental settings (Clewell et al., 2005). Clewell et al. (2005) and Covington et al. (2007) have utilized PBPK modeling coupled with rigorous statistical analyses to generate distributions of the fraction metabolized under such exposure conditions. The current work involved use of new individual and low-dose data to recalibrate the same PBPK model. Our finding of essentially the same fraction metabolized (i.e., 1%) provides additional evidence in support of the low estimates of the extent to which humans can metabolically activate minute amounts of PCE.

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Table 3.1. Summarization of kinetic data used in analysis

Reference	Measurements	Exposure	Chemical
Chiu et al. (2006)	Alveolar air concentrations Blood concentrations Urinary excretion	1 ppm	PCE TCA
Chien (1997)	Alveolar air	0.054 to 4.94 ppm	PCE
Volkel et al. (1998)	Urinary excretion	10 to 40 ppm	TCA
Monster et al. (1979)	Exhaled air concentrations Blood concentrations	72 & 144 ppm	PCE
Fernandez et al. (1976)	Alveolar air concentrations Urinary excretion	100 to 200 ppm	PCE TCA

PCE = perchloroethylene;

TCA = Trichloroethylene.

Table 3.2. Prior population mean distributions

Parameter		Natrual Scale		Log Scale	
		Mean/SD[a]	(Min[b],Max[c])	Mean/SD	(Min,Max)
BW	Body weight (kg)	70.0/12.0	(40.90,101)		
QPC	Alveolar ventilation rate (L/h/kg^(0.75))	24.0/2.04	(18.89,29.11)		
VPR	Ventilation perfusion ratio	1.45/0.16	(1.06,1.84)	0.37/0.11	(0.09,0.64)
Blood flows (fraction of cardiac output)					
QFatC	Blood flow to fat	0.05/0.01	(0.03,0.07)		
QkidC	Blood flow to kidney	0.19/0.06	(0.04,0.35)		
QLivC	Blood flow to liver	0.25/0.07	(0.07,0.43)		
QRapC	Blood flow to rapidly perfused tissue	0.19/0.05	(0.05,0.32)		
QSlwC	Blood flow to slowly perfused tissue	0.24/0.04	(0.15,0.33)		
QBrnC	Blood flow to brain	0.11/0.05	(0.01,0.24)		
Tissue volumes (fraction of body weight)					
VFatC	Fat tissue volume	0.20/0.05	(0.08,0.32)		
VKidC	Kidney tissue volume	4.40E-3/1.99E-4	(3.90E-3,4.90E-3)		
VLivC	Liver tissue volume	0.03/1.17E-3	(0.02,0.03)		
VRapC	Rapidly perfused tissue volume	0.06/0.01	(0.04,0.08)		
VSlwC	Slowly perfused tissue volume	0.46/0.10	(0.22,0.71)		
VBrnC	Brain tissue volume	0.02/0.01	(0.19E-3,0.04)		
VTCAC	TCA volume of distribution	0.09/0.03	(2.97E-4,0.17)		
PCE Partition coefficients					
PB	Blood/air			2.47/0.08	(2.27,2.66)
PFat	Fat/blood			4.68/0.19	(4.21,5.15)
PKid	Kidney/blood partition coefficient			1.60/0.14	(1.25,1.95)
PLiv	Liver/blood partition coefficient			1.63/0.14	(1.29,1.98)
PRap	Rapidly perfused tissue/blood			1.58/0.14	(1.24,1.92)
PSlw	Slowly perfused tissue/blood			1.72/0.13	(1.39,2.05)
PBrn	Brain/blood			1.57/0.15	(1.20,1.94)
Kinetics parameters					
KM	Michaelis-Meten constant (mg/l)			2.40/0.50	(1.14,3.66)
CLC	clearance (l/h/kg^0.75)			-3.38/0.68	(-5.07,-1.69)
FTCALiv	Fraction of liver PCE metabolism to TCA	0.59/0.12	(0.28,0.89)		
FTCAKid	Fraction of kidney PCE metabolism to TCA	0.77/0.11	(0.48,1.05)		
Frac	Fraction of TCA in kidney excreted in urine	0.76/0.11	(0.48,1.04)		
FracK	Fraction of liver MFO activity in kidney	0.25/0.10	(0.00,0.50)		
kUC	TCA elimination rate constant (kg^(0.25)/h)	0.05/0.01	(0.02,0.08)		

[a] Standard deviation

[b] Minimum value

[c] Maximum value

Table 3.3. Prior population variance distributions

Parameter		Prior Distribution	
		Alpha	Beta
BW	Body weight (kg)	7.25	4190
QPC	Alveolar ventilation rate (L/h/kg^(0.75))	11.3	498
Ln(VPR)	Ventilation perfusion ratio	7.68	0.74
QFatC	Blood flows to fat	6.46	0.073
QkidC	Blood flows to kidney	3.81	0.0224
QLivC	Blood flows to liver	4.15	0.0374
QSlwC	Blood flows to slowly perfused tissue	4.92	0.0579
QBrnC	Blood flows to brain	3.00	0.05
VFatC	Fat tissue volume	3.6	0.0176
VKidC	Kidney tissue volume	337	7.17
VLivC	Liver tissue volume	55.8	1.12
VRapC	Rapidly perfused tissue volume	8.23	0.109
VBrnC	Brain tissue volume	3.00	0.05
VTCAC	TCA volume of distribution	3.03	0.00727
Ln(PB)	Blood/air partition coefficient	8.68	0.752
Ln(PFat)	Fat/blood partition coefficient	5.87	0.752
Ln(PKid)	Kidney/blood partition coefficient	6.80	0.724
Ln(PLiv)	Liver/blood partition coefficient	6.89	0.735
Ln(PRap)	Rapidly perfused tissue/blood partition coefficient	6.80	0.728
Ln(PSlw)	Slowly perfused tissue/blood partition coefficient	6.81	0.741
Ln(PBrn)	Brain/blood partition coefficient	3.00	0.5
Ln(CLC)	Clearance (l/h/kg^0.75)	3.00	0.5
Ln(KM)	Michaelis-Meten constant (mg/l)	3.83	0.632
FTCALiv	Fraction of liver PCE metabolism to TCA	5.53	0.276
FTCAKid	Fraction of kidney PCE metabolism to TCA	7.78	0.811
Frac	Fraction of TCA in kidney excreted in urine	7.78	0.817
FracK	Fraction of liver MFO activity in kidney	8.24	0.755
kUC	TCA elimination rate constant (kg^(0.25)/h)	2.14	0.00202

Table 3.4. Posterior vs. prior population mean distributions and potential scale reduction values

Parameter	Natural Space				Log space				Convergence
	Prior distribution		Posterior distribution		Prior distribution		Posterior distribution		Potential scale reduction
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
BW	72.4	1.03	70.8	12.0					1.058
QPC	28.3	2.06	24.0	12.1					1.045
VPR	1.74	1.06	1.45	0.157	0.370	0.110	0.550	0.0600	1.11
QFatC	0.0507	0.00750	0.0501	0.00830					1.066
QKidC	0.278	0.0236	0.192	0.0616					1.072
QLivC	0.286	0.0227	0.250	0.0706					1.11
QSlwC	0.165	0.00810	0.243	0.0354					1.11
QBrnC	0.149	0.0387	0.110	0.0539					1.14
QRapC	0.0720	0.268	0.187	0.0539					1.066
VFatC	0.165	0.0193	0.202	0.0487					1.066
VKidC	0.00440	0.000178	0.00440	0.000199					1.072
VLivC	0.0260	0.00106	0.0260	0.00117					1.11
VRapC	0.0595	0.00659	0.0595	0.00723					1.11
VBrnC	0.0199	0.00664	0.0200	0.00723					1.14
VSlwC	0.575	0.758	0.463	0.0985					1.041
VTCAC	0.0874	0.0155	0.0869	0.0346					1.0043
PB	11.2	1.064	11.8	0.908	2.47	0.0800	2.42	0.062	1.0308
PFat	134	1.10	110	20.9	4.68	0.190	4.90	0.096	1.065
PKid	4.68	1.11	5.01	0.704	1.60	0.140	1.54	0.100	1.027
PLiv	4.76	1.10	5.17	0.718	1.63	0.140	1.56	0.0900	1.032
Prap	4.96	1.11	4.89	0.672	1.58	0.140	1.60	0.100	1.055
PSlw	5.38	1.10	5.62	0.747	1.72	0.130	1.68	0.100	1.020
PBrn	4.55	1.12	4.86	0.718	1.57	0.150	1.52	0.120	1.026
ClC	0.0251	1.17	0.0333	6.716	-3.38	0.680	-3.68	0.150	1.12
KM	12.1	1.19	12.5	6.72	2.40	0.500	2.49	0.170	1.13
FTCAKid	0.755	1.12	0.765	0.113					1.0601
FTCALiv	0.571	1.14	0.585	0.122					1.0047
Frac	0.903	1.09	0.763	0.111					1.019
FracK	0.234	1.15	0.251	0.0987					1.069
kUC	0.0405	1.14	0.0503	0.0133					1.016

Table 3. 5. Posterior variance distribution Vs. prior variance distribution

Parameter		Prior Alpha	Distribution Beta	Posterior Alpha	Distribution Beta
BW	Body weight (kg)	7.25	4190	11.6	6330
QPC	Alveolar ventilation rate (L/h/kg^(0.75))	11.3	498	16.7	1580
Ln(VPR)	Ventilation perfusion ratio	7.68	0.74	9.20	0.630
QFatC	Blood flows to fat	6.46	0.073	6.16	0.0720
QkidC	Blood flows to kidney	3.81	0.0224	8.14	0.0280
QLivC	Blood flows to liver	4.15	0.0374	9.64	0.0365
QSlwC	Blood flows to slowly perfused tissue	4.92	0.0579	15.0	0.0598
QBrnC	Blood flows to brain	3.00	0.05	3.16	0.0269
QRapC	Blood flows to quickly perfused tissue	3.6	0.0176	6.33	0.0327
VFatC	Fat tissue volume	337	7.17	4.53	0.0218
VKidC	Kidney tissue volume	55.8	1.12	347	7.37
VLivC	Liver tissue volume	8.23	0.109	55.5	1.11
VRapC	Rapidly perfused tissue volume	3.00	0.05	7.92	0.107
VSlwC	Slowly perfused tissue volume	3.03	0.00727	8.64	0.0540
VBrnC	Brain tissue volume	8.68	0.752	3.29	0.0628
VTCAC	TCA volume of distribution	5.87	0.752	4.78	0.00647
lnPB	Blood/air partition coefficient	6.80	0.724	8.065	0.700
lnPFat	Fat/blood partition coefficient	6.89	0.735	8.32	0.744
lnPKid	Kidney/blood partition coefficient	6.80	0.728	7.36	0.662
lnPLiv	Liver/blood partition coefficient	6.81	0.741	7.19	0.653
lnPRap	Rapidly perfused tissue/blood partition coefficient	3.00	0.5	6.89	0.645
lnPSlw	Slowly perfused tissue/blood partition coefficient	3.00	0.5	6.74	0.659
lnPBrn	Brain/blood partition coefficient	3.83	0.632	3.45	0.450
lnClC	Clearance (L/h/kg^0.75)	5.53	0.276	6.52	2.44
lnKM	Michaelis-Meten constant (mg/l)	7.78	0.811	7.51	0.865
FTCALiv	Fraction of liver PCE metabolism to TCA	7.78	0.817	3.83	0.402
FTCAKid	Fraction of kidney PCE metabolism to TCA	8.24	0.755	3.33	0.432
Frac	Fraction of TCA in kidney excreted in urine	2.14	0.00202	2.20	0.206
FracK	Fraction of liver MFO activity in kidney	7.25	4190	5.84	0.000435
kUC	TCA elimination rate constant (kg^(0.25)/h)	11.3	498	4.52	0.506

Table 3.6. Correlation coefficients of posterior means

	lnClC	lnKM	FTCAKid	FTCALiv	Frac	FracK	kUC	lnVPR	QPC	QFatC	QKidC.	QLivC	QSlwC	QBrnC
lnClC	1.00	-0.10	0.02	-0.02	-0.05	-0.02	-0.23	0.00	-0.10	-0.02	0.01	0.05	0.00	-0.05
lnKM	-0.10	1.00	0.00	0.01	0.00	0.02	0.05	-0.03	0.00	0.01	-0.08	0.03	-0.02	0.05
FTCAKid	0.02	0.00	1.00	-0.01	0.01	0.02	-0.02	0.00	0.02	-0.01	0.02	0.00	-0.01	-0.01
FTCALiv	-0.02	0.01	-0.01	1.00	0.02	0.01	-0.04	-0.01	-0.01	0.01	0.00	0.00	0.01	0.02
Frac	-0.05	0.00	0.01	0.02	1.00	-0.01	0.05	0.00	-0.01	0.02	-0.02	0.01	0.00	0.00
FracK	-0.02	0.02	0.02	0.01	-0.01	1.00	0.03	0.00	0.00	-0.01	-0.01	0.03	0.02	-0.02
kUC	-0.23	0.05	-0.02	-0.04	0.05	0.03	1.00	0.01	0.07	0.00	0.02	-0.02	-0.01	0.02
lnVPR	0.00	-0.03	0.00	-0.01	0.00	0.00	0.01	1.00	-0.02	0.01	0.03	-0.02	0.00	0.00
QPC.	-0.10	0.00	0.02	-0.01	-0.01	0.00	0.07	-0.02	1.00	-0.01	-0.02	0.00	-0.01	-0.01
QFatC.	-0.02	0.01	-0.01	0.01	0.02	-0.01	0.00	0.01	-0.01	1.00	0.01	0.01	0.01	-0.01
QKidC.	0.01	-0.08	0.02	0.00	-0.02	-0.01	0.02	0.03	-0.02	0.01	1.00	-0.49	-0.06	0.01
QLivC	0.05	0.03	0.00	0.00	0.01	0.03	-0.02	-0.02	0.00	0.01	-0.49	1.00	-0.02	-0.49
QSlwC	0.00	-0.02	-0.01	0.01	0.00	0.02	-0.01	0.00	-0.01	0.01	-0.06	-0.02	1.00	-0.04
QBrnC	-0.05	0.05	-0.01	0.02	0.00	-0.02	0.02	0.00	-0.01	-0.01	0.01	-0.49	-0.04	1.00
VFatC	0.01	-0.01	0.01	0.01	-0.02	0.00	0.00	-0.01	-0.04	-0.01	-0.02	-0.02	0.02	-0.02
VKidC	-0.01	-0.01	0.01	-0.01	0.01	0.00	0.01	0.01	0.00	0.01	0.01	-0.02	-0.02	0.02
VLivC.	-0.02	0.00	0.01	0.00	-0.01	0.00	0.00	-0.01	0.00	-0.01	0.01	0.00	0.00	0.01
VRapC	0.02	-0.01	0.00	-0.02	-0.02	0.00	0.00	0.01	-0.01	0.01	0.00	0.00	-0.01	0.00
VBrnC	-0.03	0.03	-0.02	-0.02	-0.01	0.00	-0.02	-0.03	0.01	-0.01	-0.03	0.02	0.01	0.00
VTCAC	-0.01	0.00	-0.01	-0.04	-0.03	0.01	0.01	0.01	0.00	0.00	-0.04	0.04	0.00	0.00
lnBW	-0.10	0.01	0.01	0.01	-0.02	0.00	0.01	-0.02	0.00	0.01	-0.02	-0.01	0.01	0.02
lnPB	-0.04	-0.01	0.00	-0.01	0.01	0.01	0.00	0.03	0.05	0.00	0.00	0.01	0.00	-0.02
lnPFat	-0.02	0.02	-0.02	0.01	0.01	0.00	0.03	-0.01	0.15	0.01	-0.01	-0.02	-0.01	0.03
lnPKid	0.04	-0.03	0.00	0.02	-0.02	0.01	0.02	0.00	0.01	0.01	-0.04	0.05	0.01	-0.02
lnPLiv	0.00	-0.04	0.01	0.00	-0.01	-0.03	0.01	0.01	0.00	-0.02	0.05	-0.06	0.01	0.03
lnPRap	0.00	-0.02	0.00	-0.02	0.00	-0.01	-0.02	0.01	0.02	0.01	0.03	0.01	0.03	-0.01
lnPSlw	-0.04	0.02	0.02	-0.01	0.03	-0.03	0.03	0.00	0.14	0.00	0.01	-0.04	0.02	-0.01
lnPBrn	0.03	-0.01	0.01	0.02	-0.02	0.01	-0.02	0.00	0.01	0.02	-0.01	0.03	0.00	-0.01

Table 3.7. Posterior predictive check

Statistic	MP	SDP
AUC	0.42	0.38
MAE	0.52	0.12

AUC = area under curve;

MAE = mean absolute error;

MP = mean of p values;

SDP = standard deviation of p values;

Table 3.8. Predicted distributions of the fraction of PCE metabolized in the liver for continuous inhalation of 1 ppb of PCE based on posteriors of model parameters

	Inhalation (1 ppb)
Mean	0.0101
Standard deviation	0.00314
Median	0.00973
5^{th} percentile	0.00498
95^{th} percentile	0.0189

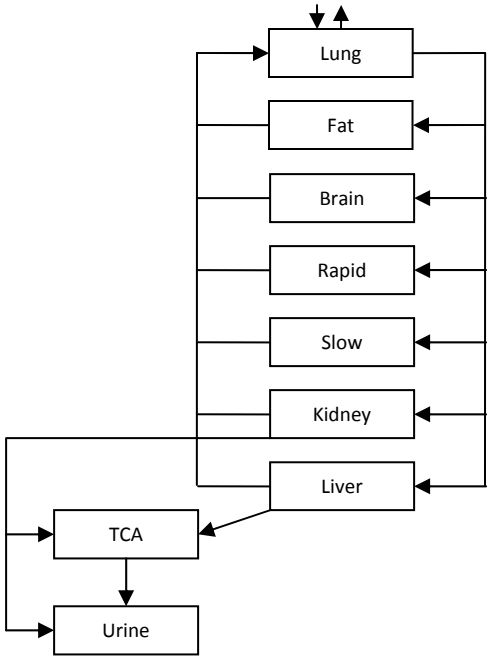

Figure 3.1. Schematic for modified PBPK model structure of Covington et al. (2007).

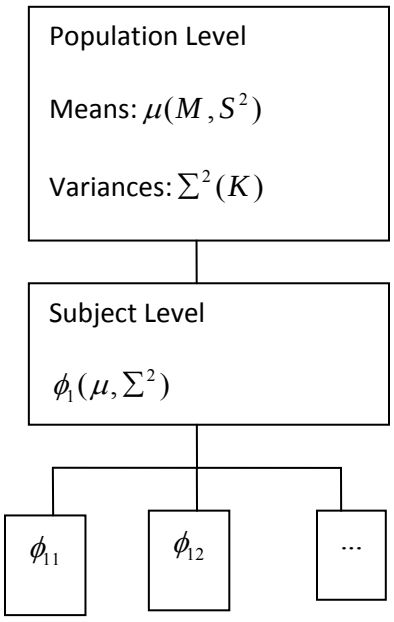

Figure 3.2. Structure of Bayesian hierarchical population model. μ and Σ^2 are prior distributions of population mean and variance. Φ_1 represents the prior distributions at subject level and its distribution parameters are randomly sampled from the distributions of population mean and population variance μ and Σ^2. Φ_{1i} (i =1,2,3,…) represents the model parameter distributions for each individual.

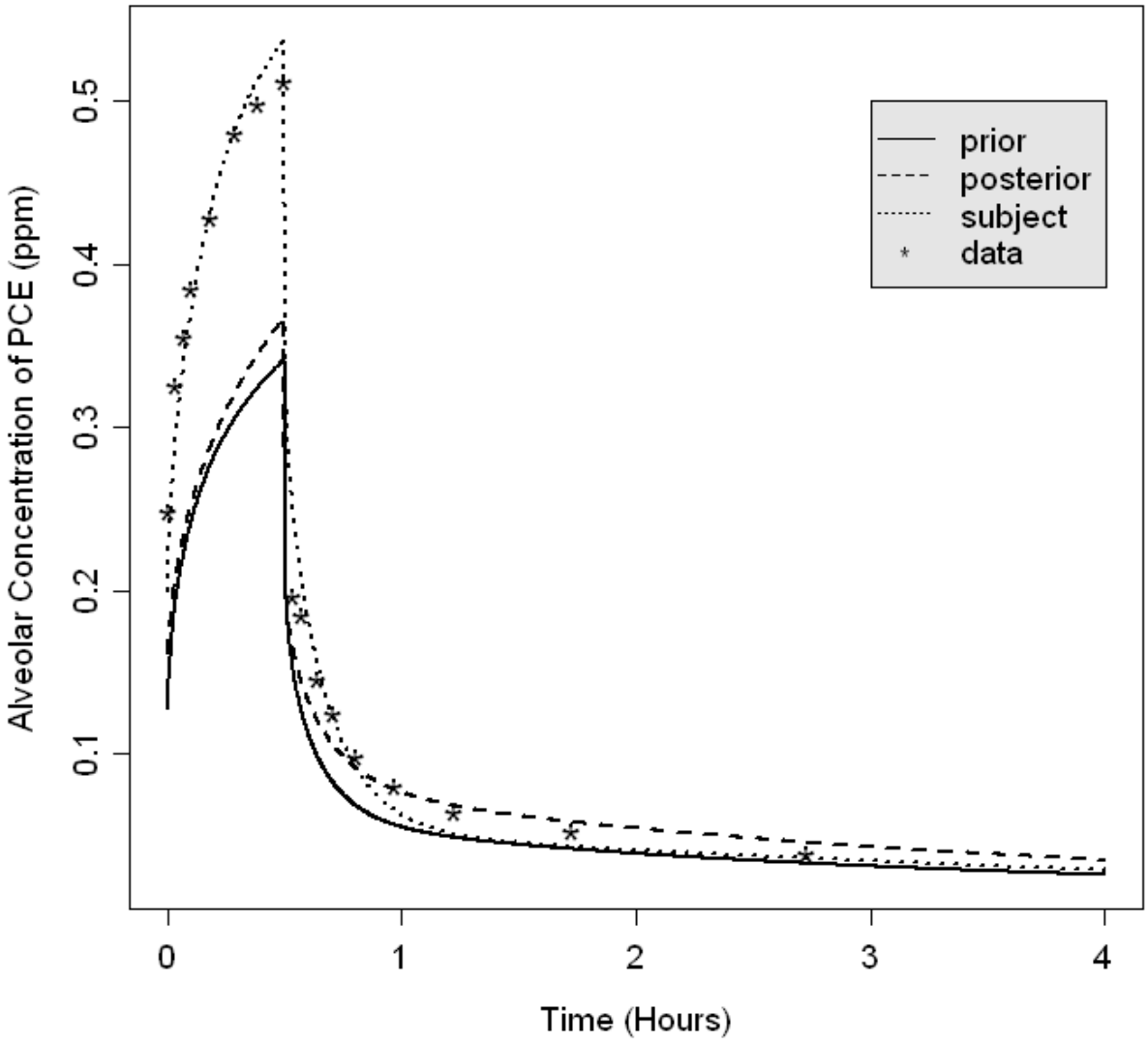

Figure 3.3. Predicted (curves) and experimental (asterisks) alveolar air concentrations of PCE for a 30-min inhalation exposure of one human subject to PCE at 1.485 ppm (Chien 1997) using prior means (solid line), population posterior means (dashed line) and the subject-specific posterior means (dotted line).

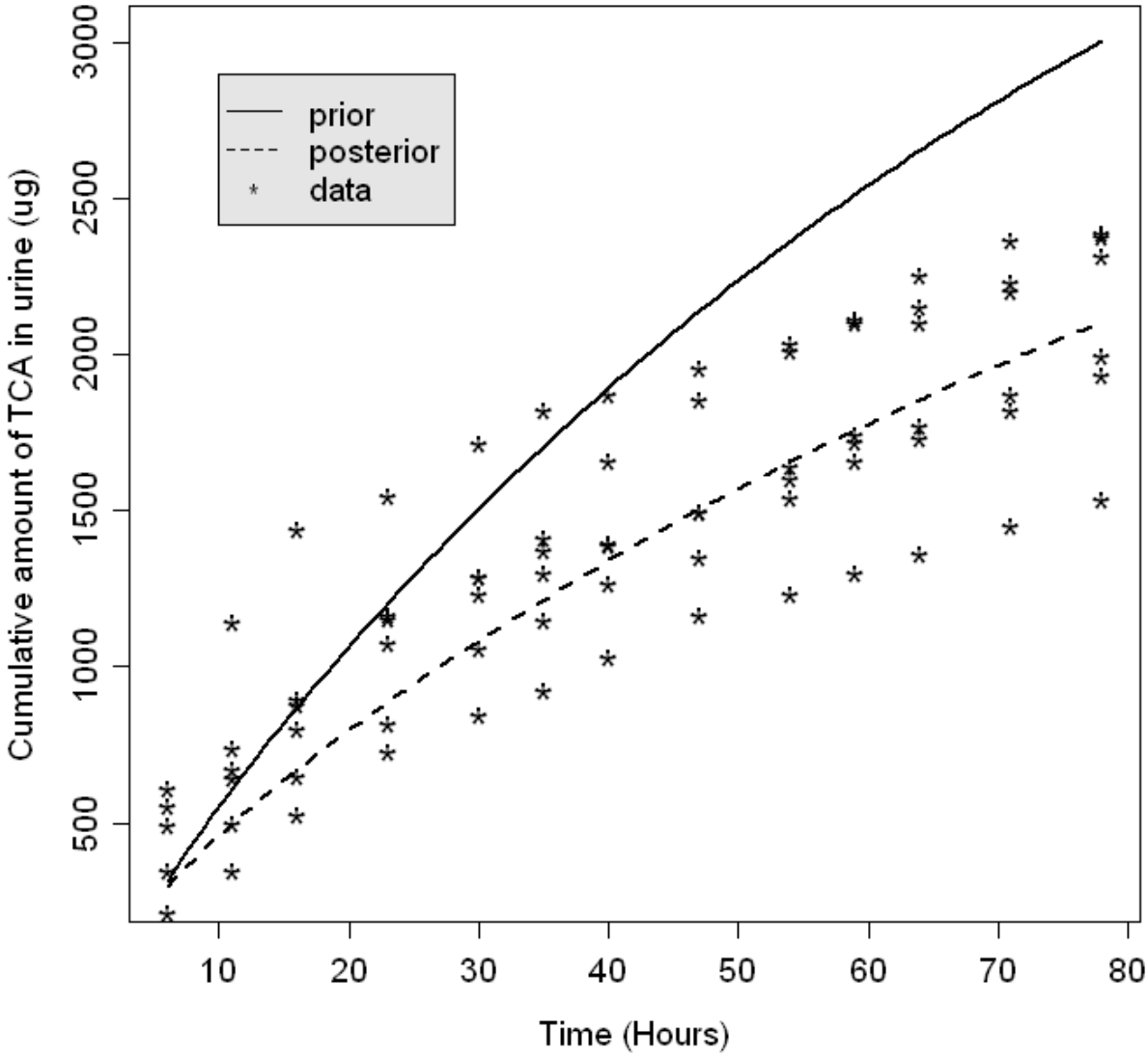

Figure 3.4. Predicted (curves) and experimental (asterisks) cumulative amounts of TCA excreted in urine for a 6-h inhalation exposure of 6 human subjects to 20 ppm PCE (Volkel et al., 1998) using prior means (solid line) and population posterior means (dashed line).

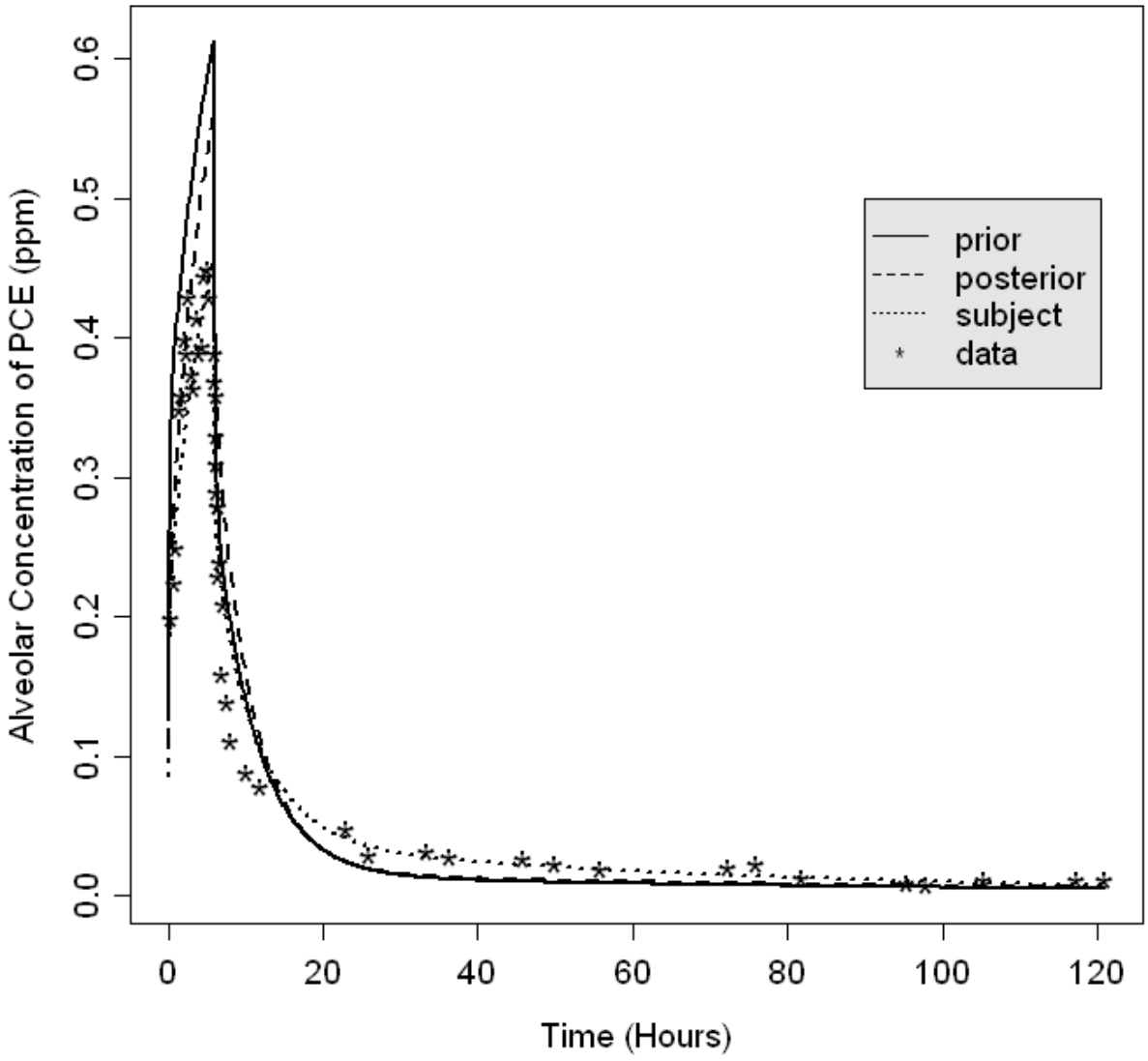

Figure 3.5. Predicted (curves) and experimental (asterisks) alveolar air concentrations of PCE for a 6-h inhalation exposure of one out of six human subjects to 1 ppm PCE (Chiu et al., 2007) using prior means (solid line), population posterior means (dashed line) and the subject-specific posterior means (dotted line).

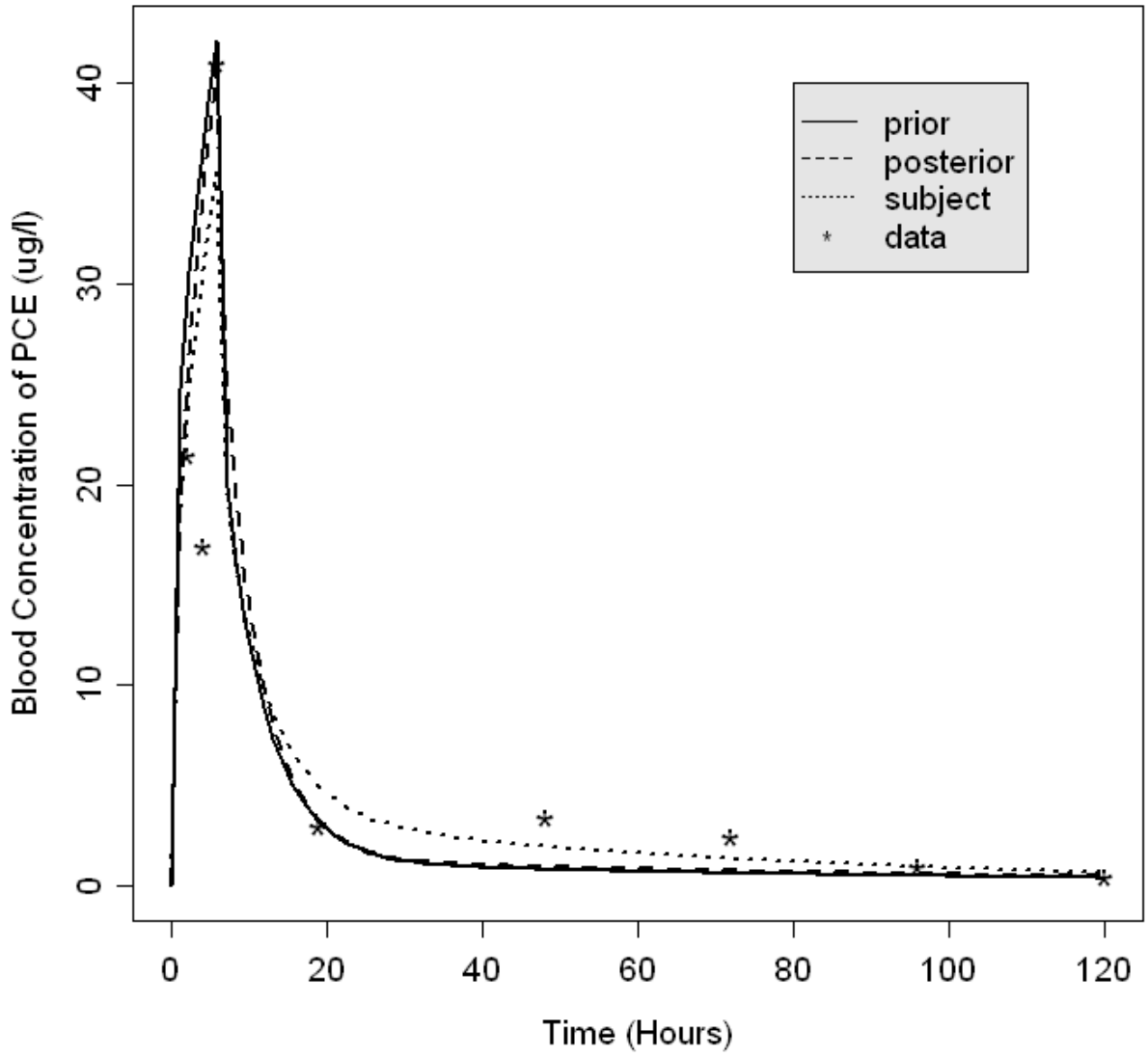

Figure 3.6. Predicted (curves) and experimental (asterisks) blood concentrations for a 6-h inhalation exposure of one out of six human subjects to 1 ppm PCE (Chiu et al., 2007) using prior means (solid line), population posterior means (dashed line) and the subject-specific posterior means (dotted line).

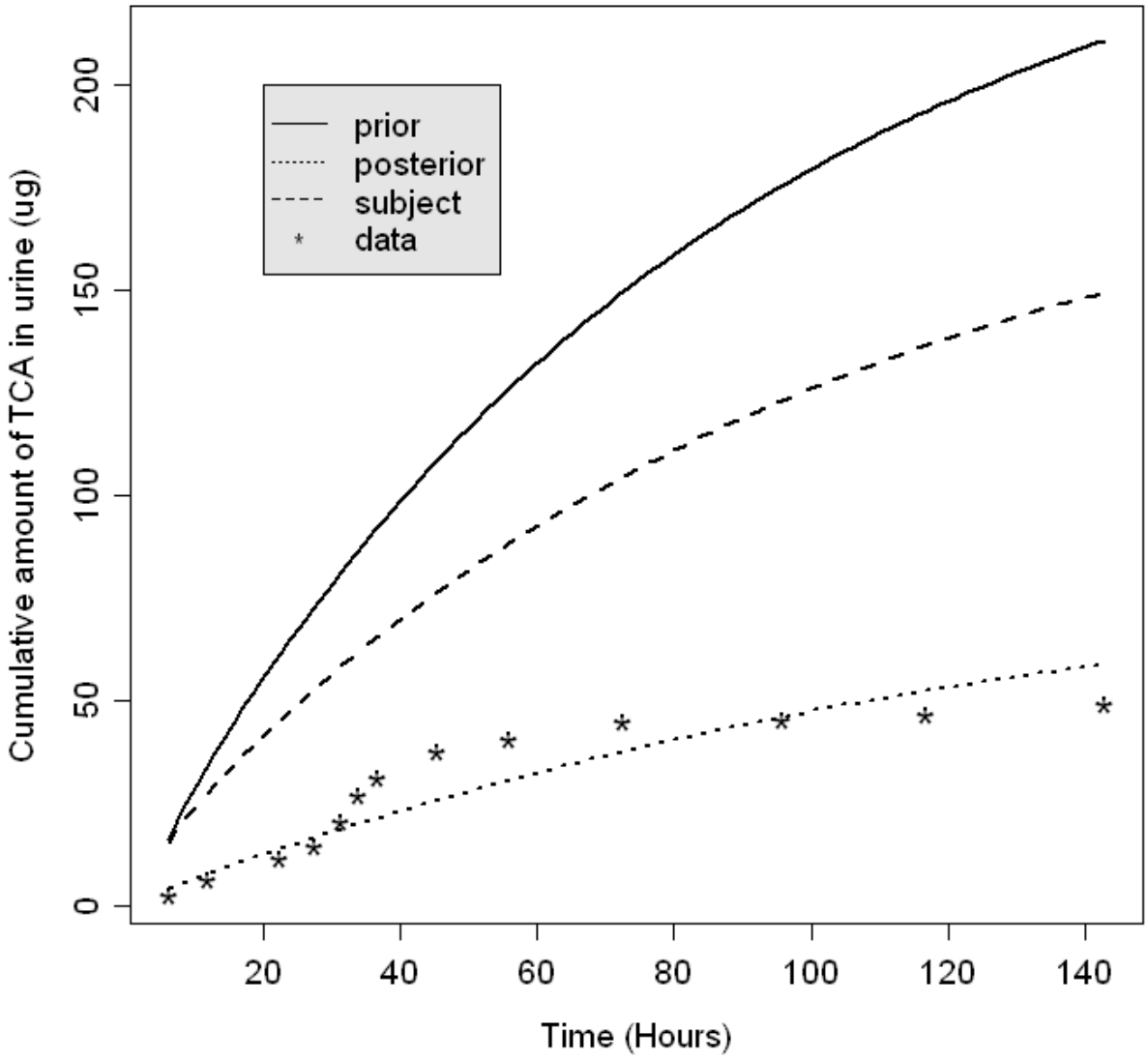

Figure 3.7. Predicted (curves) and experimental (asterisks) cumulative amounts of TCA in urine for a 6-h inhalation exposure of one out of six human subjects to 1 ppm PCE (Chiu et al., 2007) using prior means (solid line), population posterior means (dashed line) and subject-specific posterior means (dotted line).

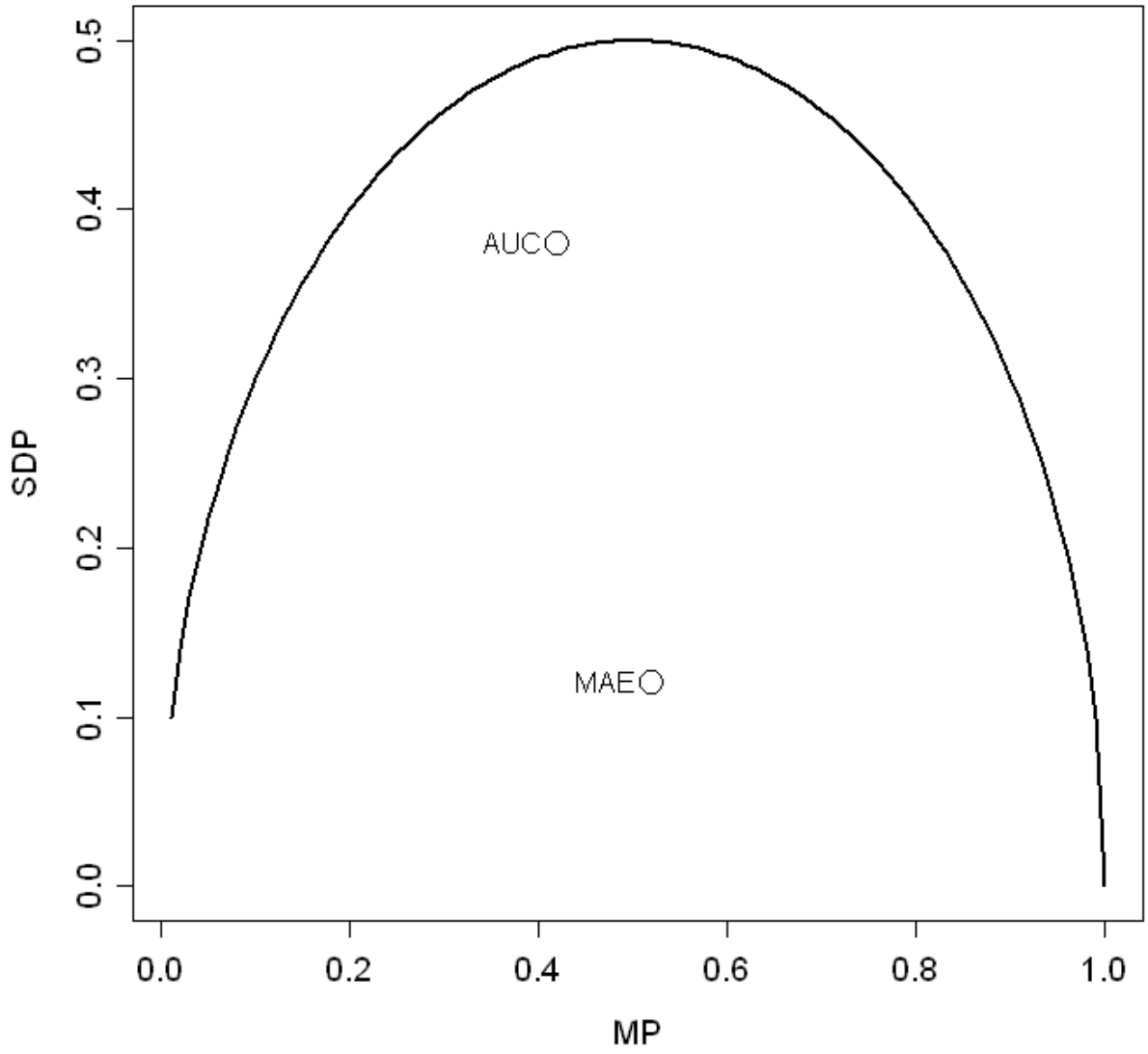

Figure 3.8. Plots of mean of p values (MP) vs. standard deviation of SDP for interest statistics area under curve (AUC) and mean absolute error (MAE) as in Table 3.6. Circles represents the points (MP, SDP) for AUC and MAE.

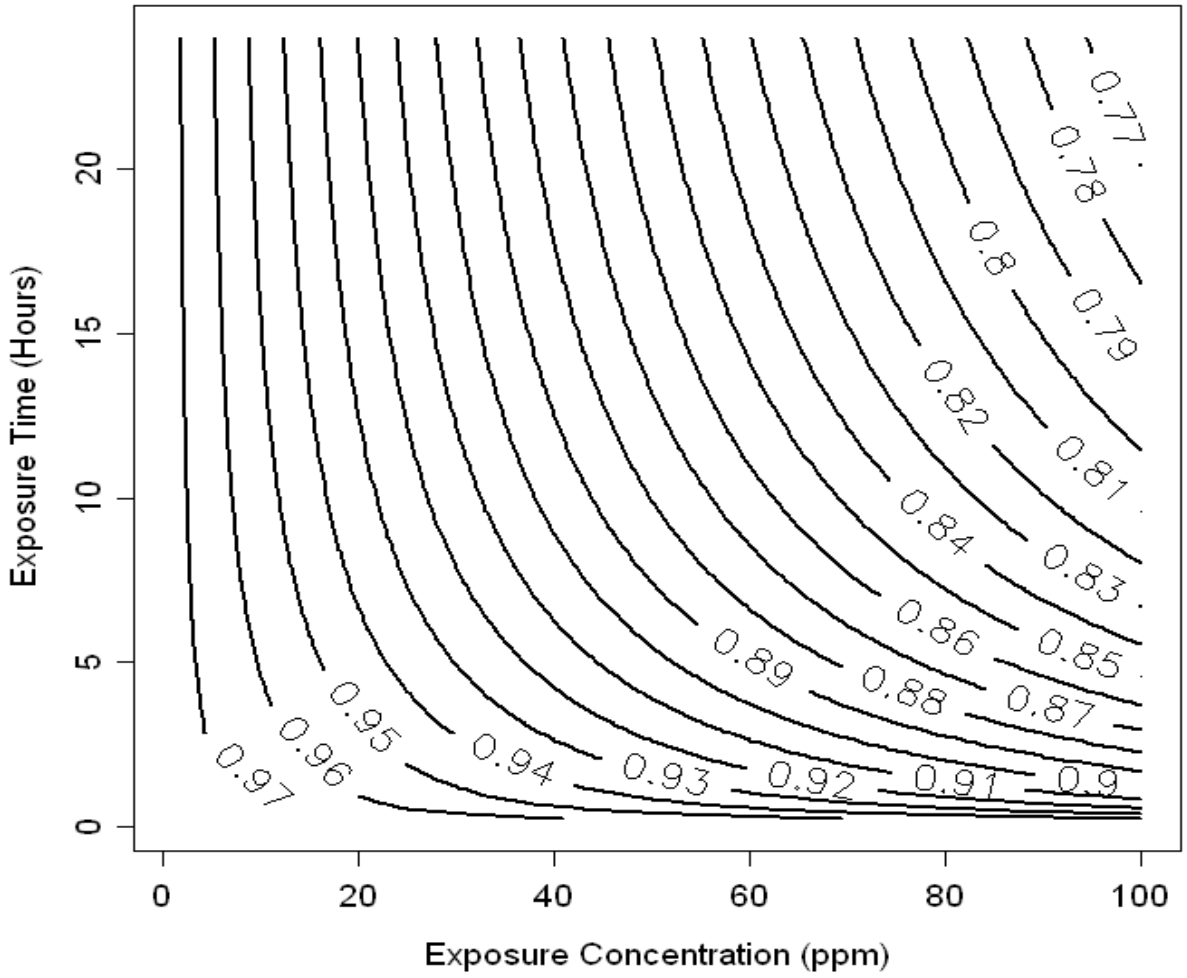

Figure 3.9. Fraction of PCE metabolized in liver under different exposure conditions.

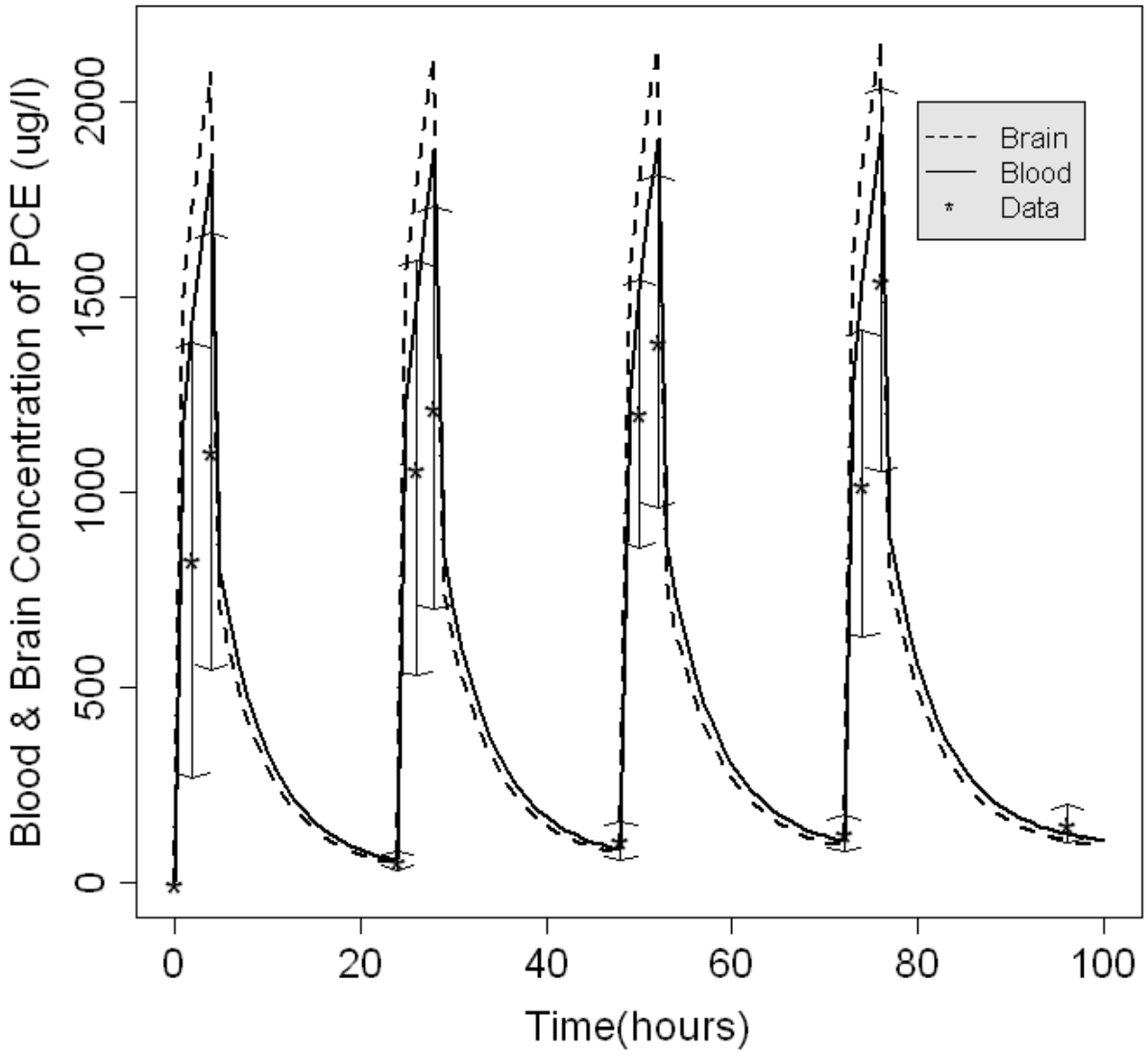

Figure 3.10. Predicted (curves) and mean (±SD) experimental (asterisks) blood and brain concentrations of PCE for a 4-h inhalation exposure of 11 human subjects to 50 ppm PCE over 4 days (Altmann et al., 1990) using population posterior means (solid line for blood and dashed line for brain).

CHAPTER 4

USE OF PHYSIOLOGICALLY BASED PHARMACOKINETICS MODEL TO RECONSTRUCT OCCUPATIONAL EXPOSURES TO PERCHLOROETHYLENE

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To be submitted to Risk Analysis

Abstract

Biomonitoring data provide information on human exposure to occupational and environmental chemicals. To reconstruct exposure profile uniquely based only on biomonitoring data seems challenging. Physiologically based pharmacokinetic (PBPK) models are powerful tools to link biomonitoring data and exposure conditions. The objective of this study was to reconstruct occupational exposures to perchloroethylene (PCE) with an combined PBPK model and exposure pattern characterization for dry cleaning workers based on sparse biomonitoring data (i.e., blood and breath concentrations of PCE before or after shift work). First, sensitivity analysis of PBPK model (Qiu et al., 2008) parameters for PCE to model outputs was conducted to identify sensitive parameters to outputs and to ascertain effects of parameter transformation on sensitivity analysis results. The variability in blood and alveolar air concentrations of PCE was shown to be more sensitive to variability in parameters ventilation perfusion ratio (VPR) and blood/air partition coefficients (PB) in either original or transformed form; the model outputs of blood TCA concentrations or urinary excretion of TCA was most sensitive to variability in parameters clearance (ClC) in either form. Values of sensitivity coefficients of log transformed parameters might exceed 1 and be difficult to explain. Curve shapes of sensitivity coefficients of model parameters over time were not affected by parameter transformation. Next, posterior distributions of exposure parameters were derived by a Bayesian approach based on exposure information available and conservative assumptions about exposure. Correlations between exposure parameters and model outputs were then calculated to detect sensitive exposure parameters. Atmospheric PCE levels in the working environment and background levels of PCE had high correlations with the biomarkers, blood and alveolar PCE concentrations. Lastly, posterior distributions of PBPK model parameters and exposure parameters were used to perform

Monte Carlo (MC) simulations. Bootstrap sampling of MC sample with respect to likelihood of model outputs was used to construct distributions of exposure profiles.

Key words**:** Perchloroethylene (PCE); occupational exposure; PBPK models; Bayesian analysis; exposure reconstruction

Introduction

Biomonitoring has been defined as a process of measuring concentrations of chemicals or their metabolites in blood, urine and tissues (Tan et al., 2005). Sometimes biomonitoring data are so sparse that samples are only collected at one time-point. Biomintoring data for a variety of chemicals has been published by U.S. Centers for Disease Control and Prevention (CDC). Sparse biomonitoring data contain less information on exposure concentration time profiles and make exposure reconstruction more difficult. Comprehensive exposure information (e.g. exposure source, route, duration, frequency, intensity; timing of collection biomomitoring data relative to exposure; human activity pattern, etc..) needs to be obtained and utilized in analyses. Biomarkers measured in biomonitoring data should be sensitive to external exposures. A previous study (Gobba et. al., 2003) demonstrated strong correlations between the cumulative external dose and blood or alveolar air concentrations of tetrachloroethylene, or perchloroethylene (PCE).

Physiologically based pharmacokinetics (PBPK) models play an important role in exposure reconstruction. PBPK models can be used to link biomarkers, such as breath, blood and urine concentrations of chemicals to exposure conditions and describe non-linear relationship between biomarkers and external exposures. For a given exposure condition, dosimetry can be determined uniquely with a PBPK model. But, based on dosimetry (biominotring data) only, exposure profiles cannot be determined uniquely.

Several exposure reconstruction methods have been developed and applied to different types of biomonitoring data. Roy and Georgopoulos (1998) used a PBPK model to reconstruct long-term inhalation exposure scenarios for volatile organic compounds (VOCs) based on biomonitoring data. In this paper, exhaled breath concentrations of benzene were identified as an appropriate biomarker of exposure to benzene. Benzene is a lipophilic chemical that accumulates in the fat compartment and reaches its steady-state after 10 days. In addition, cumulative exposures have been shown to be insensitive to exposure concentration-time profile and are proportional to the cumulative amount of the VOC in fat by assuming PBPK model is approximately linear. In this study, only cumulative exposures were estimated. The error of estimation was higher when exposure concentrations changed frequently and substantially. The estimations tended to underestimate actual exposures. Another method proposed by Tan et al. (2006) is based on Monte Carlo simulation. This method can account for variation in exposure conditions, timing between sampling and key exposure, interindividual and intraindividual dissimilarities and measurement errors. In this study, distributions of exposure and sampling parameters were defined. Monte Carlo simulations of PBPK model outputs were performed via random sampling from the predefined parameter distributions to obtain the distributions of biomarkers, such as blood concentrations of chloroform under known exposure conditions. Further, distributions of exposure conversion factor (ECFs) were derived. Finally, with observed biomonitoring data, distributions of exposure concentrations were derived. In this study, ECFs were estimated, provided there was a linear relationship between the assumed exposure conditions and model predictions. In real situations this assumption may not be satisfied. Similar methods were applied to trihalomethanes and other VOCs (Tan et al., 2007; Liao et al., 2007). Usually, biomonitoring data are collected from a large and diverse population. Sohn et al. (2004)

proposed a method based on Bayesian inference to reconstruct population-scale exposures. In this study, a PBPK model was used to predict biomarker distributions. Each model input was assigned an uncertainty distribution. Monte Carlo random sampling and Latin Hyercube sampling were recommended by Sohn et al. (2004) instead of Markov Chain Monte Carlo or Gibbs sampling. Similarly as before, maximum likelihood method was used to evaluate the concurrence between model prediction and observed biomonitoring data. The exposure pattern was characterized by the posterior distributions of exposure-related parameters. This analysis is based on complete kinetic data , not on real biomonitoring data.

PCE is an important solvent in dry cleaning operations. Dry cleaning employees are exposed to PCE mainly via inhalation while at work. Sufficiently long (8 hours/day) and high PCE (>200 ppm) exposures can potentially cause adverse health effects including hepatorenal dysfunction, neurological deficits and certain cancers (e.g., liver, kidney) (ATSDR, 1997). Neurotoxicity has become a major health concern. Changes in vision and intellectual function have been detected in dry-cleaning workers (Seeber, 1989). Biomonitoring data usually obtained by monitoring concentrations of PCE in exhaled breath and/or blood, as well as urinary excretion of TCA, the major metabolite.

Our objective is to develop a method to carry out exposure reconstructions, based on PCE occupational biomonitoring data collected by the National Institute of Occupational Safety and Health (NIOSH) (1998). For these biomonitoring data, the timings of exposure to PCE and sampling are known with reasonable certainty, but inhaled quite variable. A validated PBPK model for PCE (Qiu et al., 2008) was used to input exposure information to reconstruct exposure profiles. Sensitivity analyses of the PBPK model parameters to model outputs were performed first. Following that, a Bayesian approach using Markov chain Monte Carlo (MCMC) analysis

was used to derive posterior distributions of exposure parameters. Correlation coefficients between exposure parameters and model outputs were evaluated. Next, MC simulations were performed by sampling from distributions of PBPK model parameters and exposure parameters. Finally, exposure profiles were constructed for specific subjects by bootstrap sampling of MC samples with respect to likelihoods of model outputs.

Methods

Model structure

For dry cleaning workers, daily exposure to PCE mainly occurs via inhalation of vapors while working. Each dry cleaner may be exposed to different concentrations of PCE, even when working in the same establishment and for the same duration. Flynn (2004) proposed a stochastic differential equation for occupational exposure to VOCs and found a standard Beta distribution is the result of a probability distribution of exposures. In order to be conservative a uniform distribution which is a special case of Beta distribution family, was assumed. With assumptions for simplicity, only the inhalation pathway was incorporated into a PBPK model for PCE (Qiu et al., 2008). Parameter values [means and standard deviations (SDs)] were derived from posterior distributions of PBPK model parameters (Qiu et al., 2008) and are summarized in Table 4.1.

Sensitivity analysis

A local sensitivity analysis was performed with posterior distributions of PBPK model parameters (original form or log transformed form), to evaluate the sensitivity of response variability to each parameter while other parameters were controlled, as well as to see the effects of parameter transformation on the results of sensitivity analysis. Parameter values are chosen from the range of their distributions (mean+/-standard deviations). The selected exposure condition was inhalation of 1 ppm PCE for 6 hours via inhalation. Sensitivity coefficients were

calculated for each parameter respective to each response variable at different time-points. Therefore, the manner in which sensitivity coefficients for each parameter behaved as a function of time could be detected.

Description of biomonitoring data

Biomonitoring data utilized in this analysis was obtained from NIOSH (1998). Some 18 female dry cleaners from 4 different establishments provided data for this study. The data included alveolar air concentrations of PCE measured before and after shifts for individuals for 3 consecutive working days, as well as the PCE blood level measured before the shift on the second day. In addition, breath samples were taken either at the beginning of the work week (Monday) or immediately after the work week (Friday) for several weeks. Data obtained after the week of the 3 core shift measurements were not used to derive posteriors, but were reserved for validation. Due to these missing alveolar air concentrations and the assumption that after-shift alveolar air concentrations should be higher than before–shift alveolar air concentrations, only 8 subjects remained for future analysis. Variables of biomonitoring data collected are summarized in Table 4.2.

Derivation of distributions of exposure parameters with MCMC

In this section, a Bayesian approach was used to derive posterior distributions of exposure parameters and to reconstruct exposure profiles for dry cleaners who work for five consecutive days and have two days off. The exposure parameters were shift starting time, shift ending time, exposure level of PCE during shift and background level of PCE. For simplicity, let us assume inhaled air concentrations of PCE are constant as time weighted average (TWA) concentrations during one shift. If no personal physical and biochemical parameters specified in

the data, population posterior distributions of PBPK model parameters (Qiu et al., 2008) were used. In addition, the subjects involved in this study worked at least consecutive 2 days in order to let the amount of PCE cumulated in fat tissue approximate its steady-state.

As indicated in the biomonitoring data (NIOSH, 1998), all women involved in this study had a relatively high percentage of body fat. The mean of percentage of body fat was 39%, while the posterior mean of percentage of body fat is only 17%. Here instead of using posterior distribution of percentage of body fat, we treated its distribution as uniform with the maximum and minimum observed from data.

The prior distributions of exposure parameters were derived from observations or conservative assumptions and are summarized in Table 4.3. The PBPK model for PCE (Qiu et al., 2008) was used to describe pharmacokinetics processes in the human body upon inhalation exposure and displayed in Fig. 4.1. Values for model parameters were set at posterior population means. Markov chain Monte Carlo (MCMC) simulations were performed to derived the posterior distributions of the parameters related to exposure. Three parallel chains were run for 60,000 iterations and the last 10,000 iterations were used to derive posterior distributions of exposure parameters. Convergence of the chains were evaluated with the index of potential scale reduction according to Gelman (1996).

Correlations of exposure parameters to model outputs

Monte Carlo simulations were run with last 5,000 parameter sets from MCMC simulation outputs for 5 days a work week. Correlations between exposure parameters and model outputs were calculated at three time-points 60, 84, and 108 hours on day 3. Exposure parameters that

have high correlations with model outputs were identified. Time profiles of correlation coefficients for each exposure parameter were obtained.

Reconstruct exposure profiles

Our key interests are time-profiles of breathing-zone. A scheme for reconstructing an exposure concentration profile is presented in Fig. 4.1. First, samples from posterior distributions for exposure parameters and population means of PBPK model parameters for 10,000 times were used to construct a parameter matrix. Secondly, Monte Carlo (MC) simulations were run with parameter matrices as inputs to the PBPK model. Thirdly, outputs of MC simulations were used to evaluate the likelihood of PBPK model response variables. Finally, bootstrap sampling of 5,000 parameter vectors from the exposure parameters matrix with respect to the likelihood of model outputs was conducted.

Results

Sensitivity analysis

Initially, 8 parameters that were transformed into log form in MCMC analysis (Qiu et. al, 2008) were chosen for sensitivity analysis, in order to see the effects of parameter transformation on results of sensitivity analyses. They include clearance (ClC), affinity constant (K_M), ventilation perfusion ratio (VPR), blood/air partition coefficients (PB), fat/blood partition coefficients (PFat), liver/blood partition coefficients (PLiv), rapidly perfused tissue/blood coefficients (PRap) and slowly perfused tissue/blood coefficients (PSlw). For each model output, including alveolar air concentrations of PCE (Cal), venous blood concentrations of PCE (CVen), blood concentrations of TCA (CTCA), and urinary elimination of TCA (Urn), normalized sensitivity coefficients were calculated for the 8 parameters in original or transformed forms.

However, only parameters with sensitivity coefficients larger than 0.1 are plotted for analysis for each response variable.

For model output Cal, the results of the sensitivity analysis as shown in Fig. 4.2 indicate that parameter transformations only affect the absolute values of normalized sensitivity coefficients (NSCs), but do not affect the relative values of NSCs among the parameters. The values of NSCs for parameters in their original form are between 0 and 1 and are easy to interpret. Some values of NSCs for parameters in transformed form exceed 1 and are hard to interpret. NSCs for parameters PB and VPR in either form dominate at the beginning of exposure. NSCs for parameters PSlw and PRap in either form dominate after the end of 6- hour exposures.

For model output CVen, NSCs of parameter PB dominate over others as shown in Fig. 4.3. But NSCs of parameter VPR surge at the first hour of exposure and then drop quickly. NSCs for parameters PSlw and PRap increase during exposure and tend to stabilize after exposure. Parameter transformations affect the values of NSCs but not the shapes of curves of NSCs.

For model output CTCA, parameter clearance (ClC) in either form is the most sensitive. NSCs of parameter ClC rapidly increase at beginning of exposure and level off later as shown in Fig. 4.4. NSCs of parameter PB and parameter PF surge at the beginning and increase slowly later. For response variable Urn, parameter ClC is the most sensitive to response variable as shown in Fig. 4.5. Its NSCs rise quickly at beginning and level off. NSCs for parameter PB go up and then level off.

In all, parameter transformation makes values of NSCs exceed 1, but does not affect shape of curves of NSCs versus time. The sensitivities of model parameters are time-dependent.

Knowledge of sensitivity time-profiles for each parameter will benefit future design of experiments involving PCE exposure of humans.

Posterior distributions of exposure parameters

Convergence of three parallel chains was tested based on values of potential scale reduction. For each parameter, the value of potential scale reduction is less than 1.2. Posterior distributions of exposure parameters were derived based on the last 5,000 iterations. The posterior distributions were plotted as shown in Fig. 4.6-4.9. 95% confidence bands for exposure variables (shift starting time, shift ending time, working environmental air levels of PCE) were narrowed to days 3-5 of the week. The reason for this shrinkage is that observations are available for the last three days. Since there is no information on background levels of PCE, the width of confidence bands remain stable for a week. Simulation studies were performed to test if the posteriors of exposure parameters can give a meaningful interpretation of the data observed. The population distributions of alveolar air concentration for three work days are displayed in Fig. 4.10. Most of observations of exhaled breath concentrations of PCE fall into the 95% confidence region. Further, for a specific subject, 95% confidence bands were constructed based on individual information (subject-specific parameter values) and displayed in Fig. 4.11.

Correlation matrices

Correlation coefficients between exposure parameters and model outputs blood and alveolar air concentrations of PCE were calculated at each time point and displayed in Table 4.4-4.5. For either model output, exposure parameters, PCE levels in working environment and background levels for PCE, had high correlations with model outputs. At the early time, values of correlation coefficients are a little bit higher for each exposure parameters.

Reconstruction of occupational exposure to PCE via inhalation

The aim of this study is to reconstruct the exposure time profile for each person for each scenario. We used population information to reconstruct the unknown exposure concentration profile and linked this reconstructed part with the observed part.

For a specific subject, the reconstructed distributions of the breath concentration time profile for PCE are shown in Fig. 4.12. This graph reflects the anticipated exposure conditions for 3 weeks.

Discussion

Exposure assessment is an important part of risk assessment. For VOCs, environmental air concentrations or breathing zone concentrations of VOCs for individual subjects can be monitored. However, these measurements do not provide informations on systemic VOCs absorption. Internal biomonitoring data can reflect the amount of a VOC absorbed into body from all sources of exposure. Therefore, by use biomonitoring data and reverse dosimetry, the reliable exposure profiles can be reconstructed. In most instances, biomonitoring data that available provide very limited information on exposures, such that one cannot reconstruct precise exposure profiles. What can be expected are distributions of exposure profiles or possible exposure profiles. Monte Carlo simulations have been used to make reverse dosimetry predictions (Georgopoulos et al., 1994; Clewell et. al., 1999; Liao et al., 2007; Tan et al., 2006; Tan et al., 2007). In some studies (Liao et al., 2007; Tan et al., 2006; Tan et al., 2007), the inverse of outputs was used to generate distributions of ECFs. This method can handle large uncertainty and variability in exposure patterns. The exposure distributions of exposure are limited to concentrations of chemicals in the media and have large uncertainty. Other important exposure factors such as exposure time and exposure frequency were not considered. Thus, when

more information is available, this method is not the most desirable choice. In the present study, bootstrap sampling was used to derive distributions of sensitive exposure parameters: exposure levels and exposure durations. Advanced methods need to be developed to obtain distributions for other types of exposure parameters when sparse biomonitoring data are available. Artificial neural net work is a model independent method and has been used to develop in vitro and in vivo correlations (Dowell et al., 1997). This promising method may be used to construct distributions of exposure profile with sparse biomonitoring data.

Due to limited information from PCE biomonitoring data, we had to make some assumptions. For the occupational exposure reconstruction, we only considered temporal fluctuation of air concentrations of PCE and did not consider spatial fluctuation of air concentrations. Air PCE concentrations are assumed to be constant during a shift. After shift, background levels of PCE in air are also assumed to be constant before the next shift the following day.

The only exposure pathway here considered was inhalation, the primary route in dry cleaning shops. Other exposure pathways include ingestion of PCE-contaminated drinking water or food and dermal exposure via skin contact. These alternative routes may contribute to internal dosimetry. Occupational exposure, of course, are considerably higher than environmental exposures.

The reverse dosimetry method used in this study is dependent on the PBPK model. A PBPK model was used to link exposures and in vivo measurements of internal concentrations of PCE. The advantages of a PBPK model are its ability to integrate data from animal studies and to extrapolate from animals to humans, from one dose to another, and from one route to another

route. The disadvantages of using a PBPK model are its complex structure and the many input parameters. With increase in number of parameters, uncertainty related to model predictions also increases. Sometimes, a linear model can describe the relationship between exposure and biomonitoring. But this simple linear model may not be very useful with respect to inter-route and inter-species extrapolation. This is also a weakness of the artificial neural net work method.

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Table 4.1. Parameters used in the PBPK model for PCE

Parameters		Natural scale	
		mean	SD
BW	Body weight (kg)	70.8	12.0
QPC	Alveolar ventilation rate (L/h/kg^(0.75))	24.0	12.1
VPR	Ventilation perfusion ratio	1.45	0.16
Blood flows (fraction of cardiac output)			
QFatC	Blood flows to fat	0.050	0.0083
QKidC	Blood flows to kidney	0.19	0.062
QLivC	Blood flows to liver	0.25	0.071
QSlwC	Blood flows to rapidly perfused tissue	0.24	0.035
QBrnC	Blood flows to slowly perfused tissue	0.11	0.054
QRapC	Blood flows to brain	0.19	0.054
Tissue volumes (fraction of body weight)			
VFatC	Fat tissue volume	0.20	0.049
VKidC	Kidney tissue volume	0.0044	0.00020
VLivC	Liver tissue volume	0.026	0.0012
VRapC	Rapidly perfused tissue volume	0.060	0.0072
VBrnC	Slowly perfused tissue volume	0.020	0.0072
VSlwC	Brain tissue volume	0.46	0.098
VTCAC	TCA volume of distribution	0.087	0.035
PCE Partition coefficients			
PB	Blood/air	11.8	0.91
PFat	Fat/blood	110	20.9
PKid	Kidney/blood partition coefficient	5.01	0.70
PLiv	Liver/blood partition coefficient	5.17	0.72
Prap	Rapidly perfused tissue/blood	4.89	0.67
PSlw	Slowly perfused tissue/blood	5.62	0.75
PBrn	Brain/blood	4.86	0.72
Kinetics parameters			
KM	Michaelis-Meten constant (mg/l)	0.033	6.72
ClC	clearance (l/h/kg^0.75)	12.50	6.72
FTCAKid	Fraction of liver PCE metabolism to TCA	0.77	0.11
FTCALiv	Fraction of kidney PCE metabolism to TCA	0.59	0.12
Frac	Fraction of TCA in kidney excreted in urine	0.76	0.11
FracK	Fraction of liver MFO activity in kidney	0.25	0.099
kUC	TCA elimination rate constant (kg^(0.25)/h)	0.050	0.013

Table 4.2. Variables described in biomonitoring data (NIOSH, 1998)

Names		Type
SITE	Job site	Characteristic
JOBTITLE	Job description	Characteristic
ID	ID number	Numerical
PREBTH1-3	PCE in breath (ppm) on Core Day 1-3 (AM)	Numerical
POSTBTH1-3	PCE in breath (ppm) on Core Day 1-3 (PM)	Numerical
TWA1,2DATE	Date for collection of PCE time weighted average on Core Day 1-2	Numerical
TWA1,2	Personal PCE time weighted average (ppm) on Core Day 1-2	Numerical
DAY1,2 START	Time sampling pump switched on (24-hour clock)	Numerical
DAY1,2 STOP	Time sampling pump switched off (24-hour clock)	Numerical
PRE1-3 TIME	Time pre-shift breath sample taken (24-hour clock)	Numerical
POST1-3 TIME	Time post-shift breath sample taken (24-hour clock)	Numerical
BLOODPCE	PCE in blood (ppb)	Numerical
BLCLDATE	Date for collection of blood sample	Numerical
POSTW1-3	Post-shift breath (ppm) at end of workweek/start of weekend	Numerical
PREW1-3	Pre-shift breath (ppm) at end of weekend/start of workweek	Numerical
POSTW1 -3 CTIME	Collection time of post-shift breath (ppm) at end of workweek/start of weekend	Numerical
PREW1 -3 CTIME	Collection time of pre-shift breath (ppm) at end of weekend/start of workweek	Numerical
POSTW1-3 DATE	Date for collection of post-shift breath (ppm) at end of workweek/start of weekend	Numerical
PREW1-3 DATE	Date for collection of pre-shift breath (ppm) at end of workweek/start of weekend	Numerical

Table 4.3. Prior distributions of exposure parameters

Parameter	Distribution	Mean	SD	Upper	Lower	Source
SST(hrs)[a]	TruncNorm	7.93	0.65	6.62	9.23	Observed
SET(hrs)[b]	TruncNorm	15.8	0.60	14.7	17.0	Observed
EC(ppm)[c]	TruncNorm	2.63	1.41	1.22	4.03	Observed
BC(ppm)[d]	Uniform	-	-	0	1	Assumed

[a] Shift starting time

[b] Shift ending time

[c] Exposure levels for PCE in working environment

[d] Background levels for PCE

Table 4.4. Correlations between exposure parameters and predicted model output blood concentrations of PCE

Hours	SST[a]	SET[b]	EC[c]	BC[d]
60	-.102	-0.00147	0.527	0.278
84	-0.00290	0.0381	0.135	0.169
108	0.0190	0.00653	0.0555	0.0456

Table 4.5. Correlations between exposure parameters and predicted model output alveolar air concentrations of PCE

Hours	SST	SET	EC	BC
60	-0.0921	-0.00104	0.667	0.239
84	-0.0129	0.0324	0.110	0.153
108	0.00811	0.00913	0.0531	0.0535

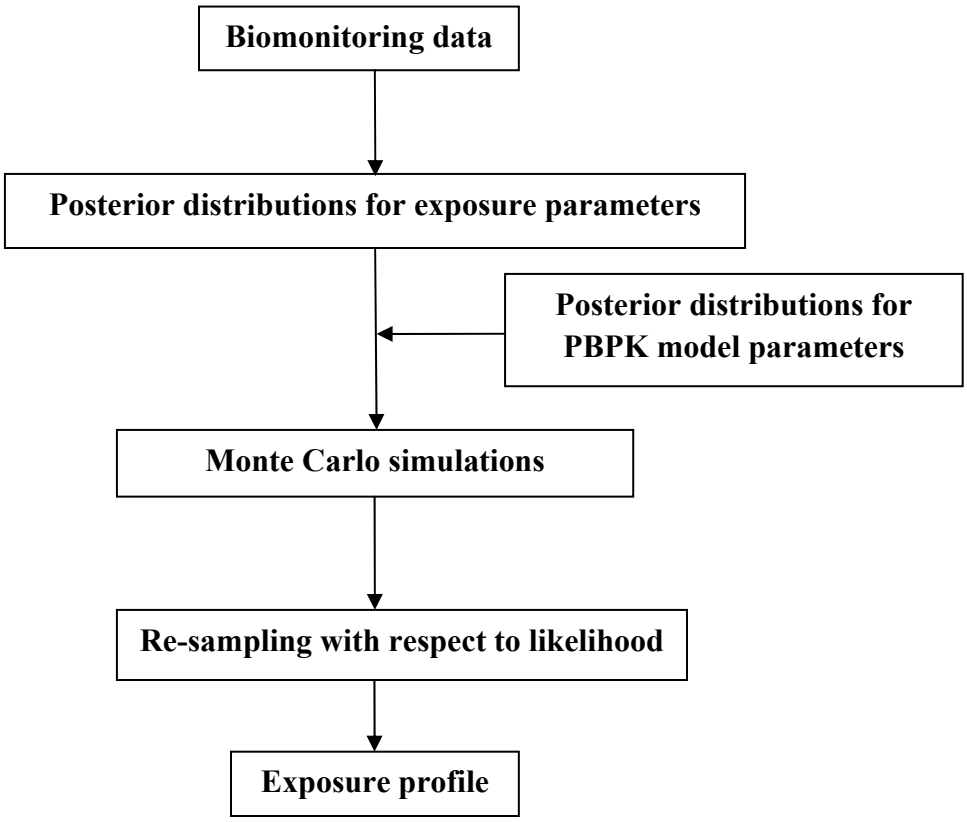

Figure 4.1. A scheme for reconstruction of exposure profile.

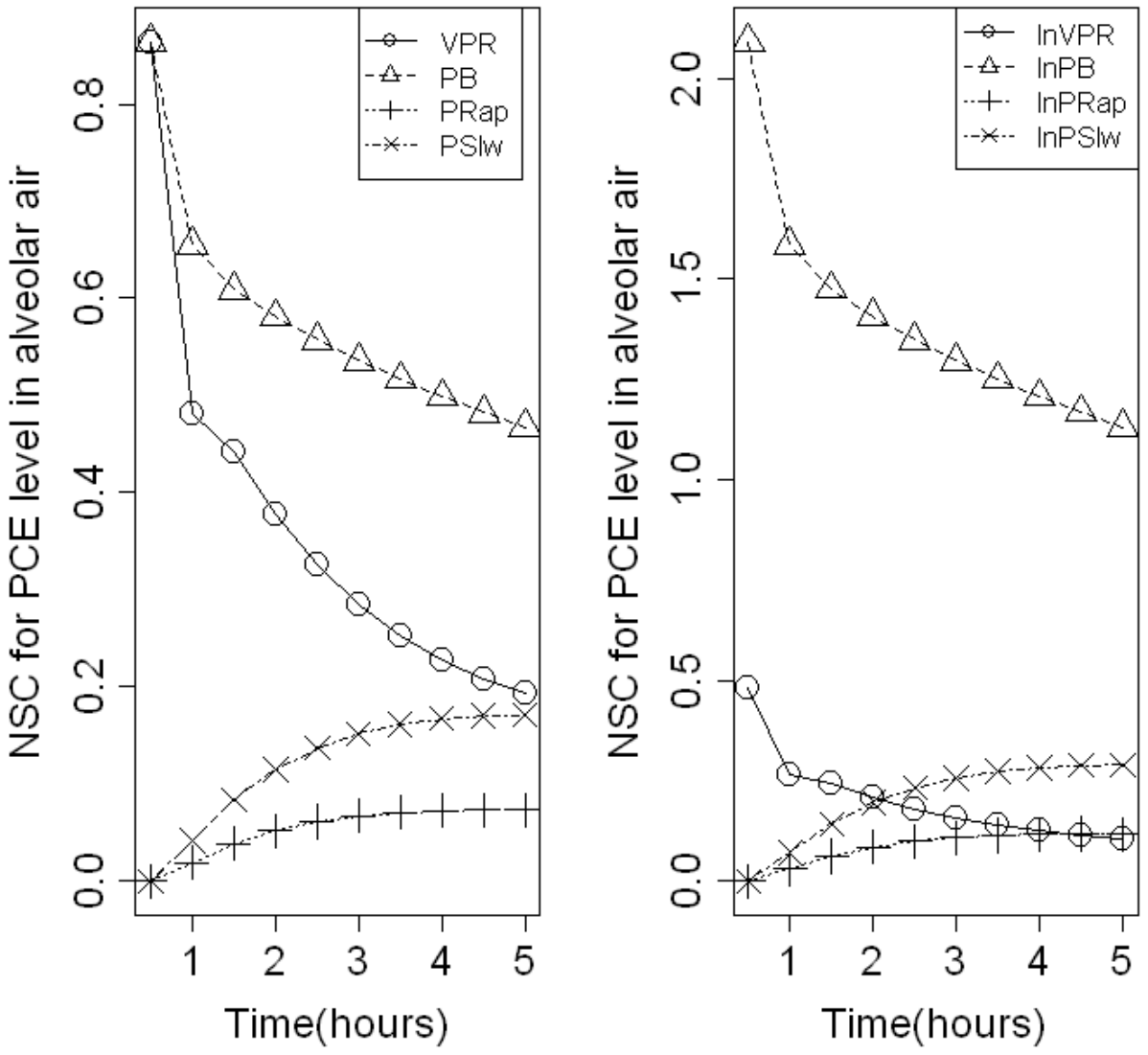

Figure 4.2. Normalized sensitivity coefficients (NSCs) for parameters log transformed in MCMC analysis. The model output is alveolar air concentrations of PCE. It was assumed that an individual was exposed to PCE at 1 ppm for 6 hours. PB: blood/air partition coefficients; VPR: ventilation perfusion ratio; PRap: rapidly perfused tissue/blood coefficients (PRap); PSlw: slowly perfused tissue/blood coefficients.

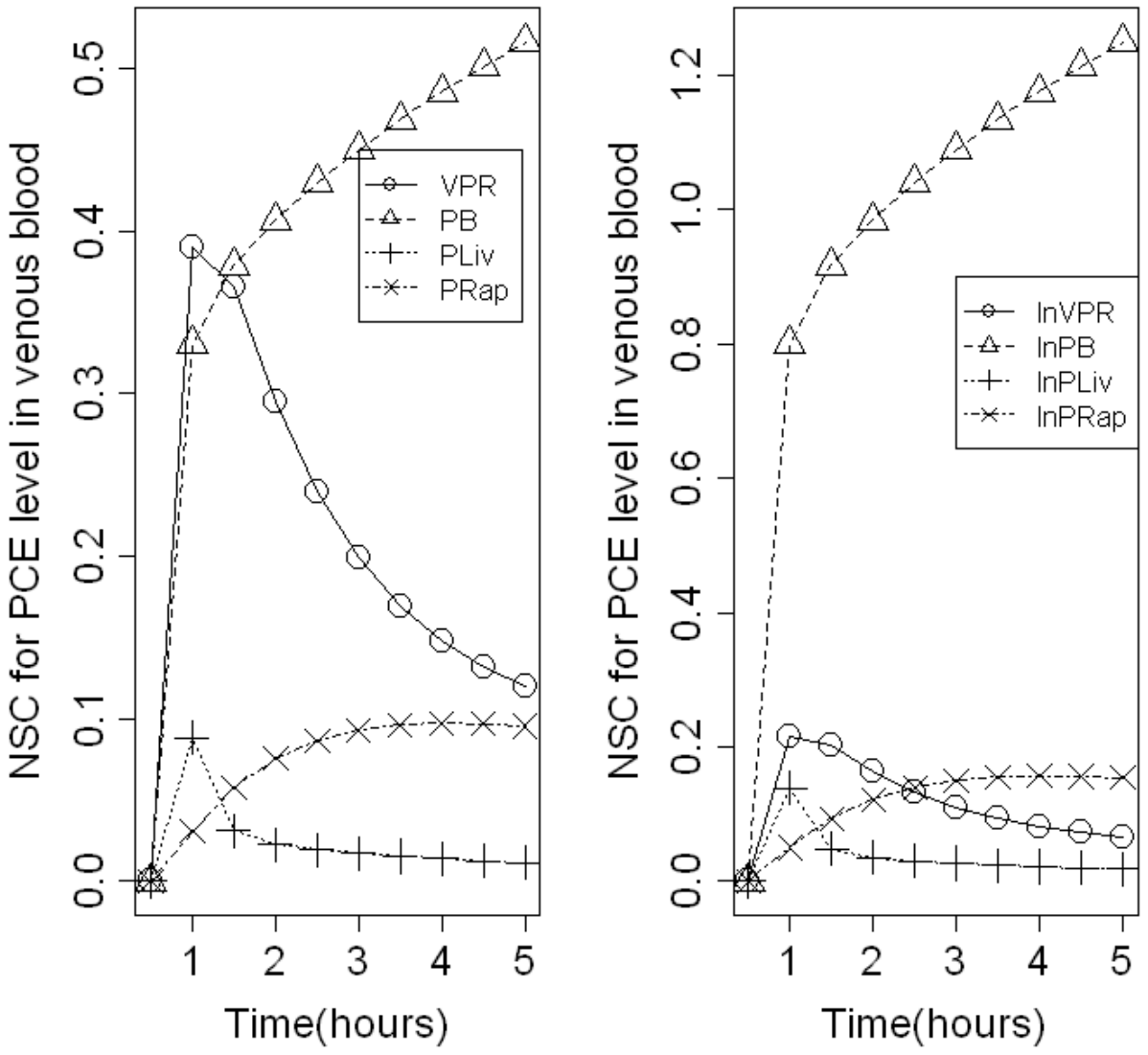

Figure 4.3. Normalized sensitivity coefficients (NSCs) for parameters log transformed in MCMC analysis. The model output is venous blood concentrations of PCE. It was assumed that an individual was exposed to PCE at 1 ppm for 6 hours. PB: blood/air partition coefficients; VPR: ventilation perfusion ratio; PRap: rapidly perfused tissue/blood coefficients (PRap); PSlw: slowly perfused tissue/blood coefficients.

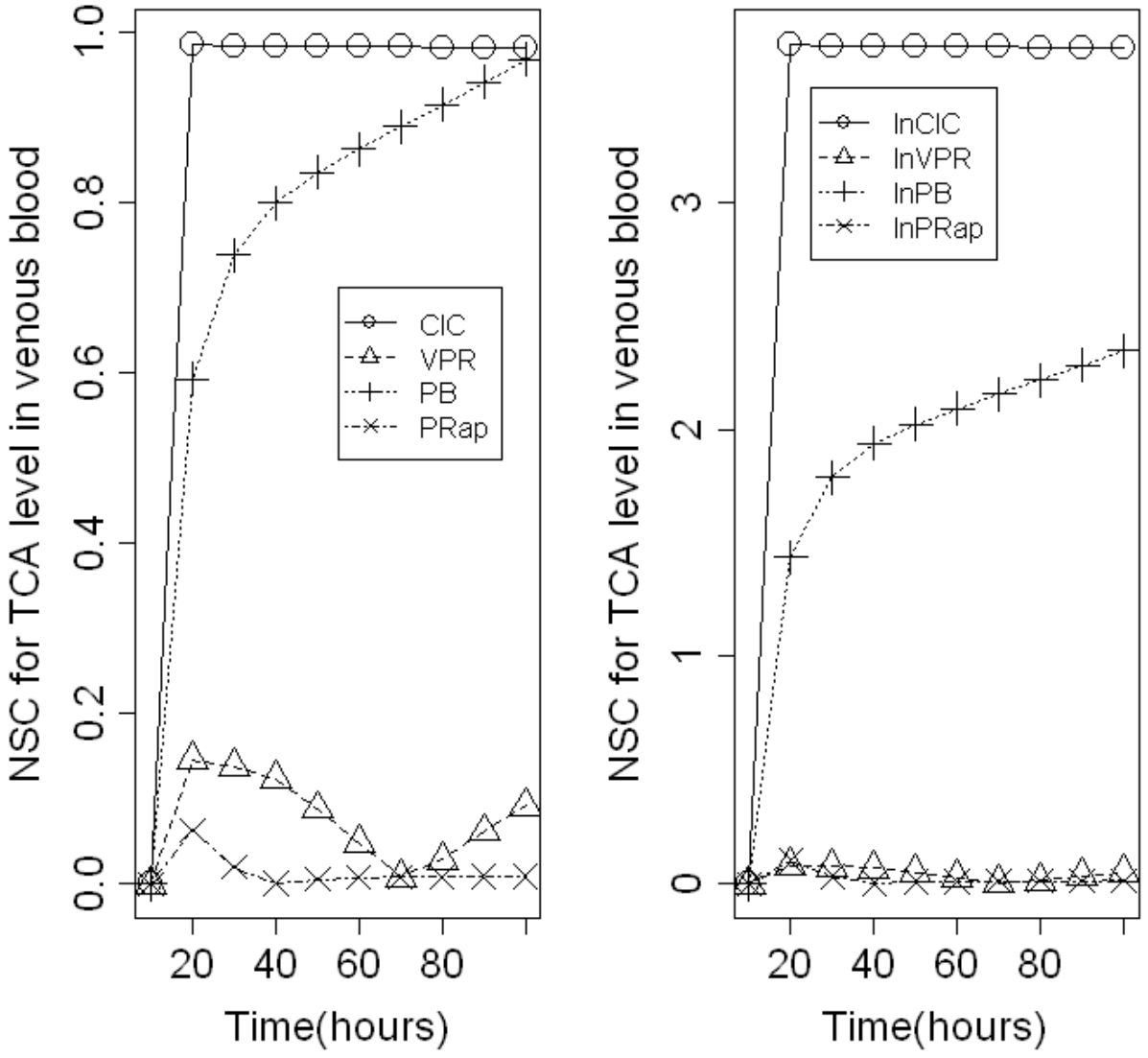

Figure 4.4. Normalized sensitivity coefficients (NSCs) for parameters log transformed in MCMC analysis. The model output is venous blood concentrations of TCA. It was assumed that an individual was exposed to PCE at 1 ppm for 6 hours. PB: blood/air partition coefficients; VPR: ventilation perfusion ratio; PRap: rapidly perfused tissue/blood coefficients (PRap); CLC: clearance.

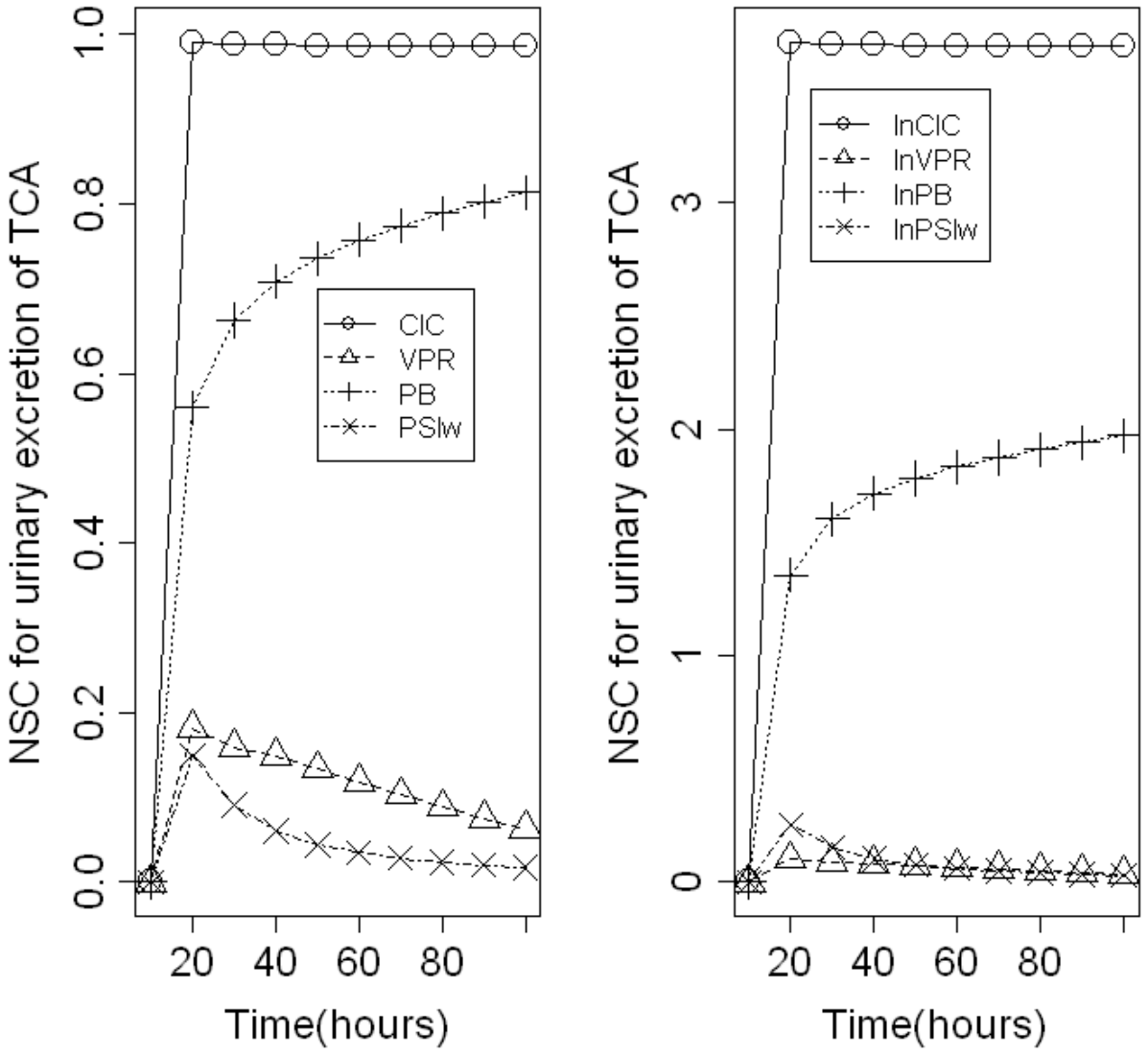

Figure 4.5. Normalized sensitivity coefficients (NSCs) for parameters log transformed in MCMC analysis. The model output is urinary excretion of TCA. It was assumed that an individual was exposed to PCE at 1 ppm for 6 hours. PB: blood/air partition coefficients; VPR: ventilation perfusion ratio; PRap: rapidly perfused tissue/blood coefficients (PRap); CLC: clearance.

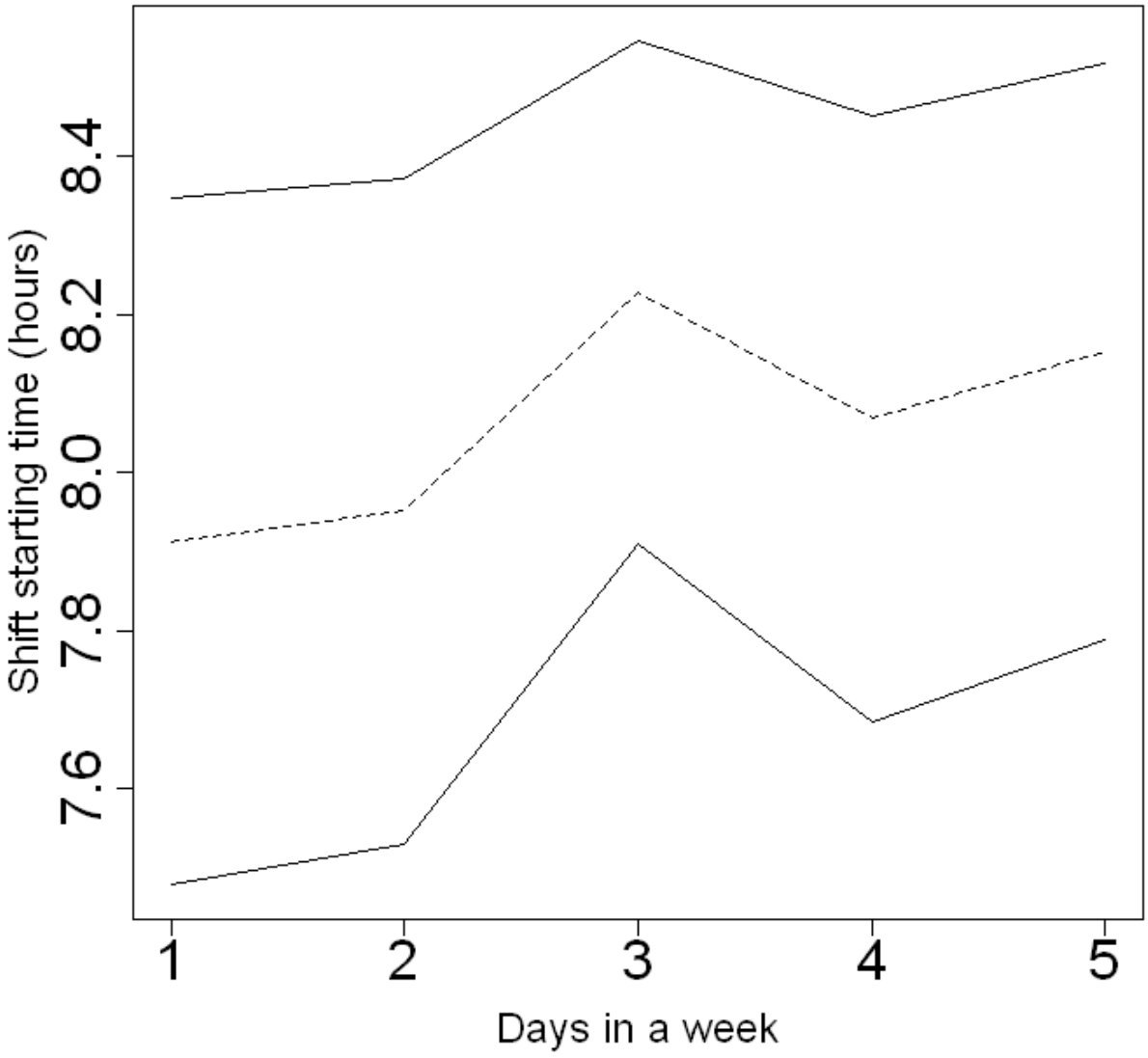

Figure 4.6. Posterior distributions of shift starting time for each work day in a week. 95% upper and lower bound (solid lines) and mean (dash line) of shift starting time are displayed.

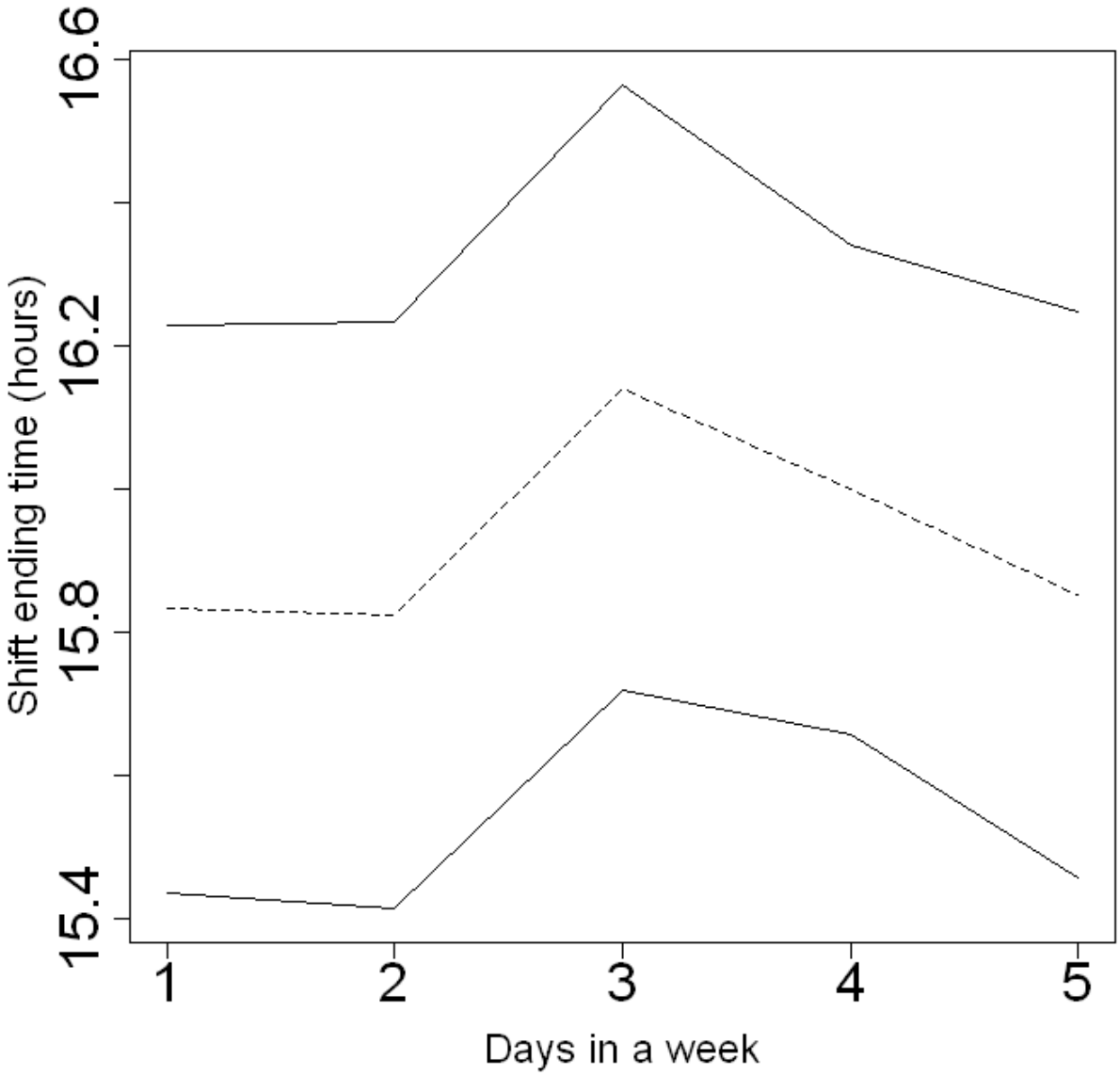

Figure 4.7. Posterior distributions of shift ending time for each work day in a week. 95% upper and lower bound (solid lines) and mean (dash line) of shift ending time are displayed.

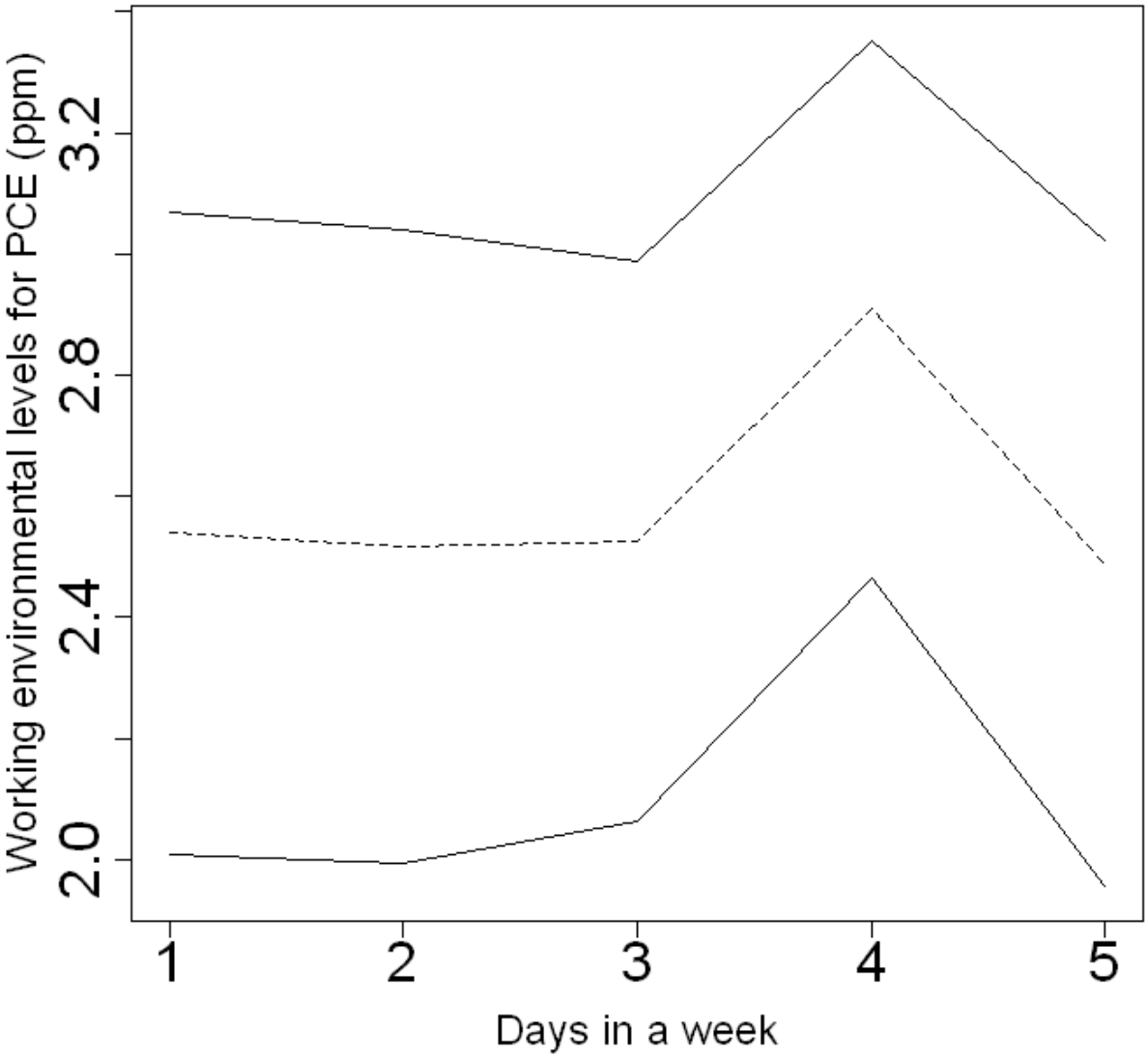

Figure 4.8. Posterior distributions of levels for PCE in working environments for each work day in a week. 95% upper and lower bound (solid lines) and mean (dash line) of levels for PCE in working environments are displayed.

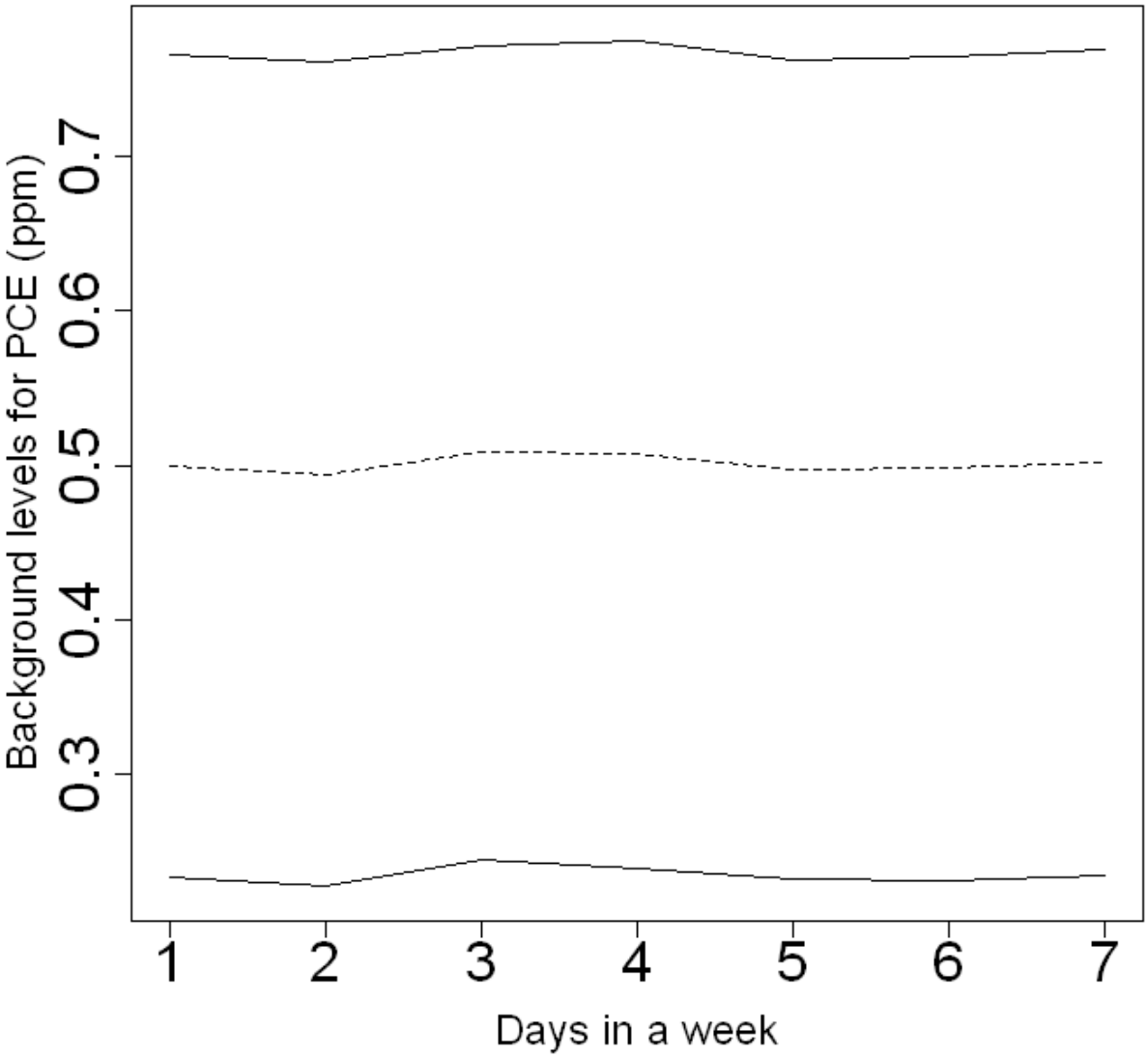

Figure 4.9. Posterior distributions of background levels for PCE for each day in a week. 95% upper and lower bound (solid lines) and mean (dash line) of background levels for PCE are displayed.

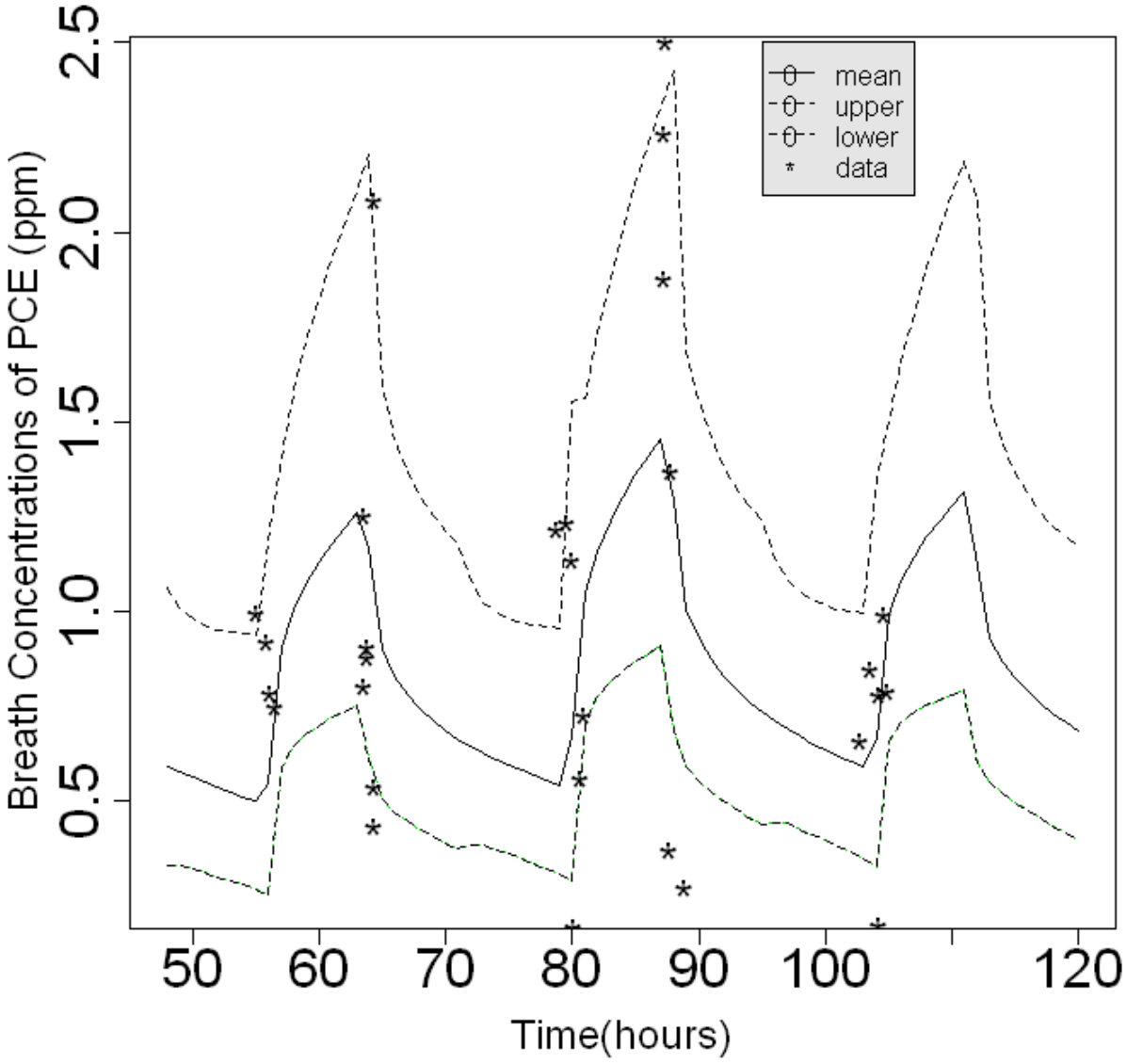

Figure 4.10. Population distributions of breath concentrations of PCE (curves) and observed data (symbol) are displayed for 3 work days.

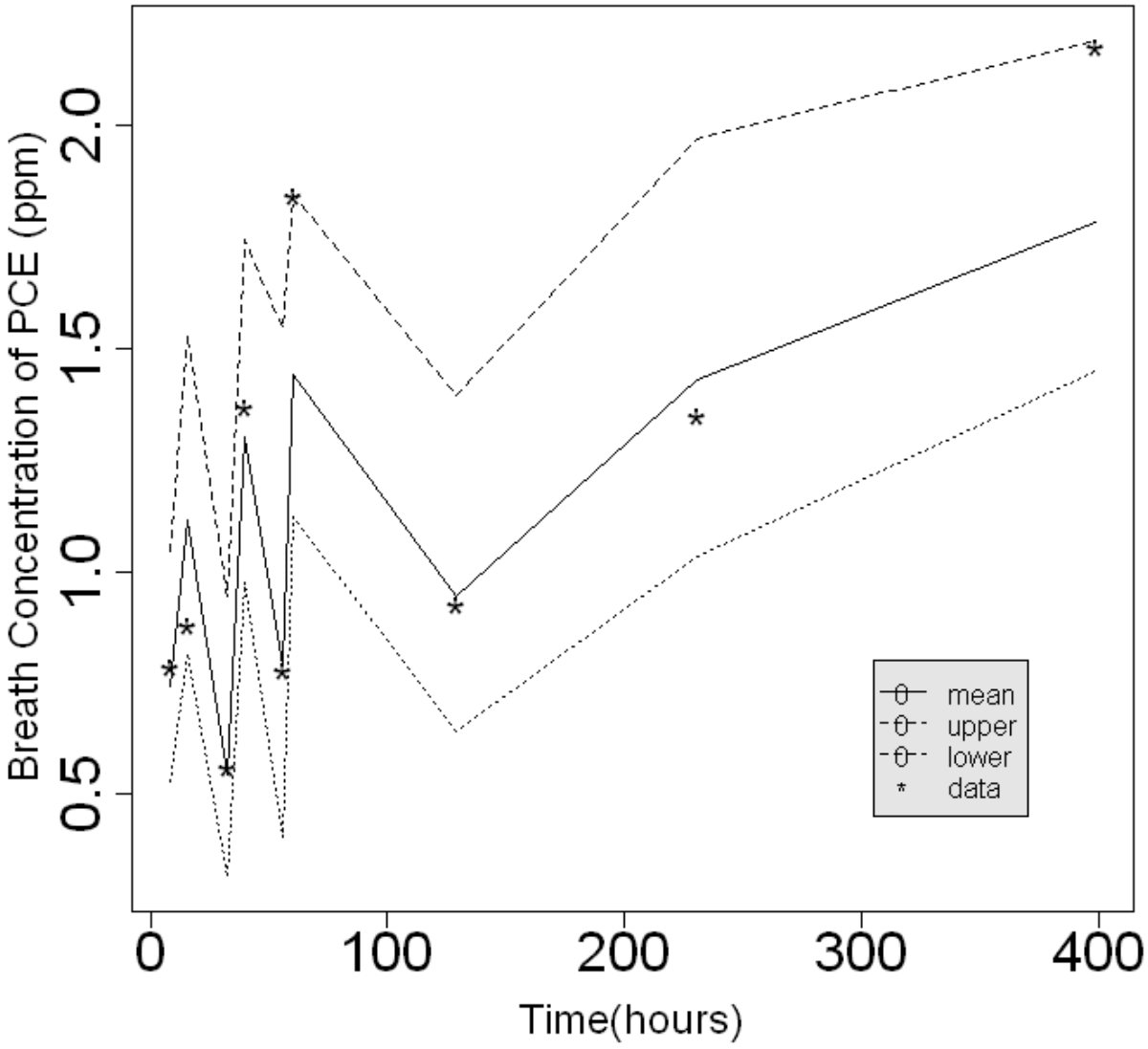

Figure 4.11. Distributions of breath concentrations of PCE (curves) and observed data (symbol) for one subject are displayed for 3 weeks.

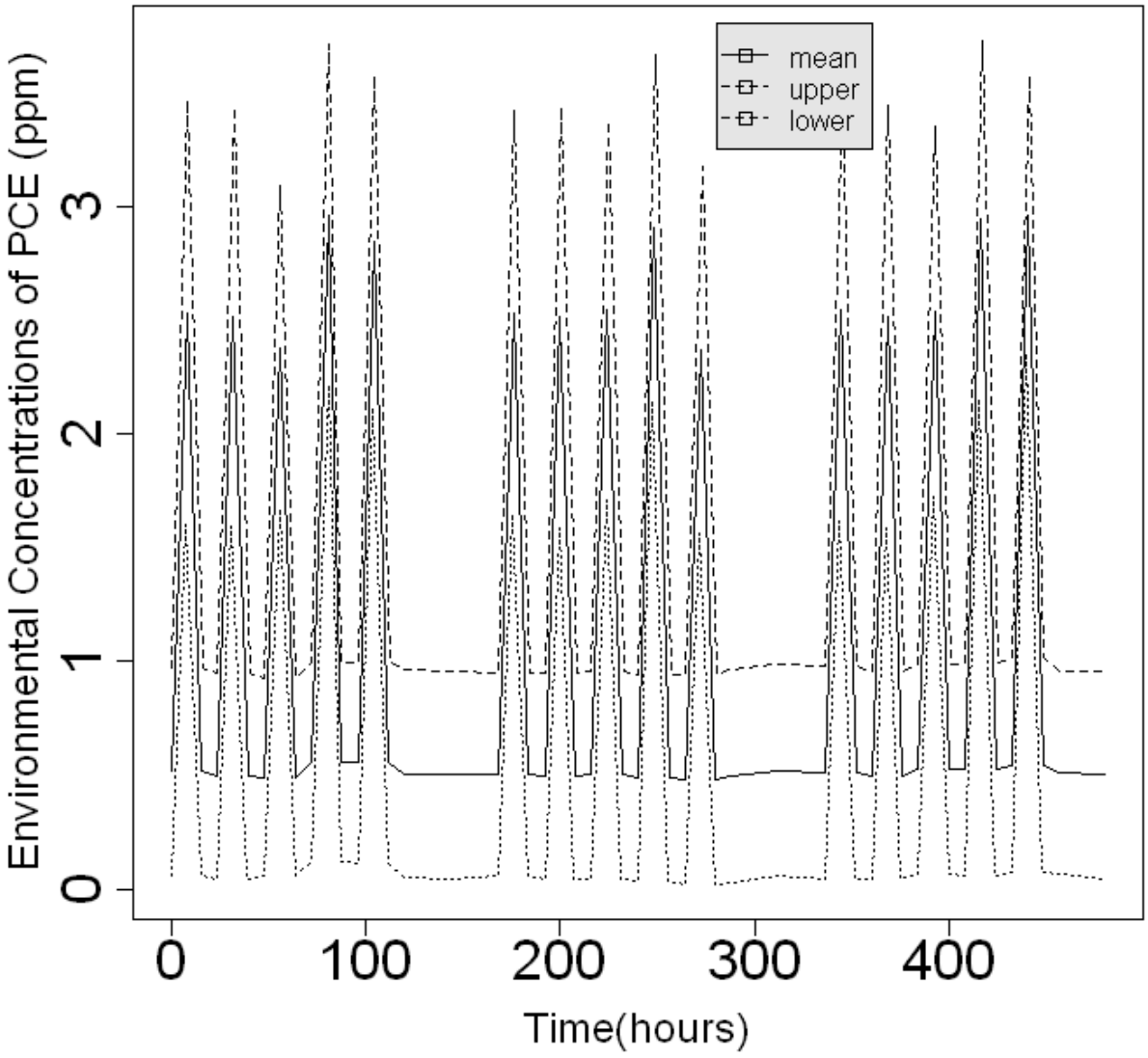

Figure 4.12. Reconstructed distributions of environmental concentrations of PCE for one subject (the same as in Figure 4.11) were displayed as 95% upper and lower bound (dash lines) and mean (solid) for three working weeks.

CHAPTER 5

CONCLUSIONS

Bayesian Analysis of PBPK model

One purpose of this study is to derive population distributions of PBPK model parameters for PCE via a Bayesian approach. Markov chain Monte Carlo simulations with a PBPK model integrated with a hierarchical population model based on kinetic data and prior distributions of model parameters. The second purpose of this study is to make use of posterior distributions of model parameters to predict the fraction of PCE metabolized in liver under different exposure conditions. As exposure time and exposure levels for PCE decrease, the fraction of PCE metabolized in liver tends to stabilize at around 1%. In addition, the upper 95th percentile for fraction of PCE metabolized in liver at a concentration of 1 ppm was reliably estimated to be 1.89%. The relationship between brain concentration of PCE and visual evoked potentials was described by a nonlinear model. The low value supports estimates of some other research groups and provides additional evidence that the capacity of humans to metabolically activate low levels of PCE to cytotoxic/carcinogenic metabolites is very limited.

Exposure Reconstruction

Another purpose of this dissertation is to reconstruct exposure profiles based on biomonitoring data. First, sensitivity analysis of PBPK model parameters to model outputs was performed and effects of parameter transformation on sensitivity analysis were described. The variability in blood and alveolar air concentrations of PCE was shown to be more sensitive to variability in parameters ventilation perfusion ratio (VPR) and blood/air partition coefficients (PB) in either original or transformed form; the model outputs of blood TCA concentrations or

urinary excretion of TCA was most sensitive to variability in parameters clearance (ClC) in either form. Values of sensitivity coefficients of log-transformed parameters might exceed one and be difficult to explain. Curve shapes of sensitivity of model parameters over time were not affected by parameter transformation. Distributions of exposure parameters were derived with a Bayesian approach based on biomonitoring data. Monte Carlo simulations were performed, based on distributions of PBPK model parameters and exposure parameters. Following that, re-sampling of Monte Carlo simulation outputs with respect to likelihood was used to reconstruct exposure profile distributions. These distributions reflect the intensity and duration of exposure and help evaluate safety under this exposure profile.

Future Work

Several aspects that need to be improved were uncovered during this research. First, the PBPK model for PCE needs to be validated further with kinetic data for the major metabolite, TCA. The PBPK model plays a key role in prediction of important indices for risk assessment of PCE. The PBPK model used in the current analysis was based on an assumption of PCE metabolized in kidney. Further, mechanistic and kinetic studies are needed to validate this assumption. Secondly, more kinetic data at low PCE exposure levels are needed to adjust distributions of PBPK model parameters to their true distributions. Thirdly, kinetic data including brain concentrations of PCE are needed from animals studies. More robust methods need to be developed to deal with the sparse biomonitoring data.

APPENDICES

APPENDIX A

HUMAN PBPK MODEL FOR PERCHLOROETHYLENE

Program: PCE.model

#Modified by Junshan Qiu

#Date Modified: 01/08/07

#Last Changed: 05/08/07

#Based on the model of Covington TR, Gentry PR, Van Landingham CB, Andersen ME, Kester #JE, Clewell HJ. 2007. The use of Markov chain Monte Carlo uncertainty analysis to support a #Public Health Goal for perchloroethylene. Regul. Toxicol. Pharmacol. 47, 1-18.

```
# PCE.Model

States = {
          ARap, #Amount of PCE in rapidly tissue (mg);
          ASlw, #Amount of PCE in slowly tissue (mg);
          AFat, #Amount of PCE in fat tissue (mg);
          AKid, #Amount of PCE in kidney tissue (mg);
          ALiv, #Amount of PCE in liver tissue (mg);
          ABrn, #Amount of PCE in brain tissue (mg);
          ATCA, #Amount of TCA (mg);
        AUrnTCA, #Amount of TCA in urine (mg);
          AExh, #Amount of PCE exhaled (mg);
        AMetLiv, #Amount of PCE metabolized in liver (mg);
        AMetKid, #Amount of PCE metabolized in kidney (mg);
        InhDose  #Amount of PCE inhaled (mg);
         };


Inputs = {
      Conc #Concentration of PCE inhaled (ppm);
         };

Outputs = {
     zAUrnTCA, #Amount of TCA excreted in urine (ug)
         CVen, #Venous blood concentrations of PCE (ug/l)
         CTCA, #Venous blood concentrations of TCA (ug/l)
      CAlvPPM, #Alveolar air concentrations of PCE (ppm)
      TotDose, #Total amount of PCE intaked (mg)
    TotTissue, #Total amount of PCE in tissue (mg)
     TotMetab, #Total amount of PCE metabolized (mg)
   MassBalPCE, #Mass balance of PCE (mg)
   MassBalTCA  #Mass balance of TCA (mg)
};

#Global parameters
      BW = 70.0;          # Body Wt (kg)
    lnBW = 4.25;          # Body Wt (kg)

# Flow Rates (L/hr/kg**0.75)
     QPC = 24.0;          # Cardiac output
  lnVPR = 0.372;          # QPC/QCC
```

```
# Fractional Blood Flows to Tissues (fraction of cardiac output)
        QFatC = 0.05;           #Fat
        QKidC = 0.18;           #Kidney
        QLivC = 0.23;           #Liver
        QSlwC = 0.25;           #Slowly perfused tissues
        QBrnC = 0.11;           #Brain

# Fractional Tissue Volumes (fraction of BW)
        VTCAC = 0.84;           # volume of body to which TCA is distributed
        VFatC = 0.21;           # Fat
        VKidC = 0.0044;         # Kidney
        VLivC = 0.026;          # Liver
        VRapC = 0.0595;         # Rapidly perfused tissues
        VSlwC = 0.44;           # Slowly  perfused tissues
        VBrnC = 0.02;           # Brain

# Partition Coefficients for PCE (from covington)
         lnPB = 2.44;          # Blood/air
       lnPFat = 4.83;          # Fat/blood
       lnPKid = 1.62;          # Kid/blood
       lnPLiv = 1.66;          # Liver/blood
       lnPRap = 1.62;          # Rapidly perfused/blood
       lnPSlw = 1.81;          # Slowly perfused/blood
       lnPBrn = 1.58;          # Bain/blood (from Dr. Chien, 1997)

# Molecular Weights
        MWPCE = 165.8;         # PCE
        MWTCA = 163.5;         # TCA

# TCE Metabolism Constants according to Tammie
        lnClC = -3.31;        # VMaxC/KM (L/hr/BW**0.75)
         lnKM = 2.04;         # Oxidative affinity (mg/L)
           KM = 7.7;          # Oxidative affinity (mg/L)
        lnkUC = -3.77;        # TCA elimination rate(kg**0.25/hr)
          kUC = 0.023;        # TCA elimination rate(kg**0.25/hr)
     FTCALiv = 0.6;           # Fractional split of TCE to TCA in liver
     FTCAKid = 1.0;           # Fractional split of TCE to TCA in kidney
        Frac = 1.0;           # Fraction of TCA in kidney excreted in urine;
       FracK = 0.1;           # Fraction of liver MFO activity in kidney;

# Exposure parameters
         Conc = 0.0;          # Inhalation exposure conc.(ppm)

# Stoichiometry
   StochTCATCE = MWTCA / MWPCE;

# These parameters have to be global so they can be used in the
# dynamic section after scaling.  The calculations in this global
# section will be superceded by those in the initialize section.
#Blood flow
    QC = 1;
    QP = 1;
  QFat = 1;
  QKid = 1;
  QLiv = 1;
  QRap = 1;
  QSlw = 1;
```

```
  QBrn = 1;

#Tissue volume (fraction of body weight)
  VFat = 1;
  VKid = 1;
  VLiv = 1;
  VRap = 1;
  VSlw = 1;
  VBrn = 1;
  VTCA = 1;

#PCE partition coefficients (Based on Covington)
     PB = 11.58;
   PFat = 125.2;
   PKid = 5.06;
   PLiv = 5.28;
   PRap = 5.06;
   PSlw = 6.11;
   PBrn = 4.86;   # from Dr. Chien thesis
   VMax = 1;
TotConc = 1;

#Statistical Parameters for population variances
#kinetics parameters
V_ClC = 1;
V_KM = 1;
V_kUC=1;
V_FTCALiv=1;
V_FTCAKid=1;
V_Frac=1;
V_FracK=1;
V_VPR=1;

#Blood Flow

V_QPC = 1;
V_QFatC = 1;
V_QKidC = 1;
V_QLivC = 1;
V_QSlwC = 1;
V_QBrnC = 1;

#Volume
V_VFatC = 1;
V_VKidC = 1;
V_VLivC = 1;
V_VRapC = 1;
V_VBrnC = 1;
V_VTCA=1;

#partition coefficients
V_BW = 1;
V_PB = 1;
V_PFat = 1;
V_PKid = 1;
V_PLiv = 1;
V_PRap = 1;
```

```
V_PSlw = 1;
V_PBrn = 1;

V_zAUrnTCA = 1;
V_CAlvPPM=1;
V_CVen = 1;
V_CTCA=1;
V_CMixExhPPM = 1;
kU=1;
################### End of Global Parameter section #################

Scale {

      BW75 = pow(BW, 0.75);
      BW25 = pow(BW, 0.25);

# Scaled Flow Rates (L/hr)

          QP = QPC * BW75;
          QC = QP/exp(lnVPR);

# Blood Flows to Tissues (L/hr)

        QFat = QFatC * QC;
        QKid = QKidC * QC;
        QLiv = QLivC * QC;
        QSlw = QSlwC * QC;
        QBrn = QBrnC * QC;
        QRap = QC - QFat - QKid - QLiv - QSlw - QBrn;

# Tissue Volumes (L)

        VFat = VFatC * BW;
        VKid = VKidC * BW;
        VLiv = VLivC * BW;
        VSlw = VSlwC * BW;
        VBrn = VBrnC * BW;
        VTCA = VTCAC*BW;
        VRap = 0.85*BW - VFat - VKid - VLiv - VSlw - VBrn;

# Volumes of Distribution

      # VTCA = VTCAC * BW;
#partition coefficients
    PB = exp(lnPB);
  PFat = exp(lnPFat);
  PKid = exp(lnPKid);
  PLiv = exp(lnPLiv);
  PRap = exp(lnPRap);
  PSlw = exp(lnPSlw);
  PBrn = exp(lnPBrn);   # from Dr. Chien thesis

# PCE Metabolism Constants
          KM = exp(lnKM);
        VMax = exp(lnClC)*KM*BW75;
        Frac = exp(lnFrac);
#Initial states
```

```
          ARap = 0.0;
          ASlw = 0.0;
          AFat = 0.0;
          AKid = 0.0;
          ALiv = 0.0;
          ABrn = 0.0;
          AExh = 0.0;

       AMetLiv = 0.0;
       AMetKid = 0.0;
       InhDose = 0.0;

            ATCA=0.0;
       AUrnTCA = 0.0;

};
###################### End of Initialization ########################

Dynamics{


#*************************************************************************
**
#***                        TCE Model
***
#*************************************************************************
**

# Inhalation Concentration (mg/L)
        CInh = Conc*MWPCE/24450.0;

# Venous Concentrations (mg/L)
       CVRap = ARap/VRap / PRap;
       CVSlw = ASlw/VSlw / PSlw;
       CVKid = AKid/VKid / PKid;
       CVFat = AFat/VFat / PFat;
       CVLiv = ALiv/VLiv/PLiv;
       CVBrn = ABrn/VBrn/PBrn;

# Concentration of PCE in mixed venous blood (mg/L)

        CVen = (QFat*CVFat + QLiv*CVLiv +QKid*CVKid + QSlw*CVSlw + QRap*CVRap
+ QBrn*CVBrn) / QC;

# Concentration in arterial blood (mg/L)

        CArt = ((QC * CVen) + (QP * CInh)) / (QC + (QP/ PB));

# Concentration in alveolar air (mg/L)

        CAlv = CArt / PB;
     CAlvPPM = (CAlv < 1.0e-15 ? 1.0e-15 : CAlv * (24450.0 / MWPCE));

# Amount exhaled (mg)
       dt(AExh) = QP* CAlv;

# Concentration in mixed exhaled air (mg/L)
```

```
     CMixExh = (0.7 * CAlv) + (0.3 * CInh);
  CMixExhPPM = (CMixExh * 24450.0) / MWPCE;

# Amount of PCE in rapidly perfused tissues (mg)
        dt(ARap) = QRap * (CArt - CVRap);

# Amount of PCE in slowly perfused tissues
        dt(ASlw) = QSlw * (CArt - CVSlw);

# Amount of PCE in fat tissue (mg)
        dt(AFat) = QFat*(CArt - CVFat);

# Amount of PCE in brain tissue (mg)
        dt(ABrn) = QBrn*(CArt - CVBrn);

# Amount of PCE metabolized in liver (mg)
        RAMetLiv = (VMax * CVLiv) / (KM + CVLiv);
     dt(AMetLiv) = RAMetLiv;
       AMetLivBW = AMetLiv / BW;


# Amount of PCE in liver (mg)
        dt(ALiv) = (QLiv * (CArt - CVLiv))- RAMetLiv;
            CLiv = ALiv / VLiv;

# Amount of PCE metabolized in kidney (mg)
        RAMetKid = (VMax * CVKid*FracK) / (KM + CVKid);
     dt(AMetKid) = RAMetKid;
       AMetKidBW = AMetKid / BW;

# Amount of PCE in kidney (mg)
        dt(AKid) = (QKid * (CArt - CVKid))-RAMetKid;
            CKid = AKid / VKid;

# Mass Balance for PCE
# Total intake from inhalation (mg)
        RInhDose = QP * CInh;
     dt(InhDose) = RInhDose;

         TotDose = InhDose;
       TotTissue = ABrn + ARap + ASlw + AFat + ALiv + AKid;
        TotMetab = AMetLiv + AMetKid;
      MassBalPCE = TotDose - TotTissue - TotMetab - AExh;


#*************************************************************************
**
#***                        TCA Sub-model
***
#*************************************************************************
**
#Amount of TCA produced from liver and kidney outside of urine
      RATCA =(RAMetLiv*FTCALiv )*StochTCATCE-ATCA*kU + (1-
Frac)*RAMetKid*FTCAKid;
   dt(ATCA) = RATCA;
       CTCA = ATCA/VTCA;
```

```
# Amount of TCA in urine (mg)
       dt(AUrnTCA) = ATCA*kU + Frac*RAMetKid*FTCAKid;
        MassBalTCA = (AMetLiv*FTCALiv )*StochTCATCE -ATCA- AUrnTCA;

};
################ End of Dynamics ####################################


CalcOutputs{
 zAUrnTCA = (AUrnTCA < 1.0e-15 ? 1.0e-15 : AUrnTCA*1000);
 CTCA = (CTCA < 1.0e-15 ? 1.0e-15 : CTCA*1000);
 CVen = (CVen < 1.0e-15 ? 1.0e-15 : CVen*1000);
 CMixExhPPM=(CMixExhPPM < 1.0e-15 ? 1.0e-15 : CMixExhPPM);
 CAlvPPM=(CAlvPPM < -1.0e-15 ? 1.0e-15 : CAlvPPM);
};
```